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PROVISIONAL APPLICATION COVER SHEET

Mail Stop Provisional Patent Application

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53(c).

Docket Number 8940.6144		Type a plus sign (+) inside this box →	
INVENTOR(s)/APPLICANT(s)			
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TITLE OF INVENTION (500 characters max)			
NOVEL HUMAN cDNA CLONES AND METHODS OF THEIR USE			
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ENCLOSED APPLICATION PARTS (check all that apply)			
<input checked="" type="checkbox"/> Specification	461 Pages	<input type="checkbox"/> Small Entity Statement	
<input type="checkbox"/> Drawing(s)	____ Sheets ____ Figures	<input type="checkbox"/> Other (specify)	
METHOD OF PAYMENT (check one)			
<input type="checkbox"/> A check or money order is enclosed to cover the Provisional filing fees		PROVISIONAL FILING FEE	
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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

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☐ Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

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UNITED STATES PROVISIONAL PATENT APPLICATION

for

NOVEL HUMAN cDNA CLONES AND METHODS OF  
THEIR USE

by

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**BACKGROUND OF THE INVENTION****Field of the Invention**

[001] The present invention is related generally to novel human cDNA clones and novel polypeptides encoded therefrom, and their compositions. The polynucleotides and polypeptides are useful in diagnostic and therapeutic applications for a variety of diseases and conditions. The present invention also relates to methods of modulating a biological activity through the use of the novel polynucleotides and novel polypeptides of the invention.

**Description of Related Art**

[002] Sequencing of the genomes, or portions of the genomes, of numerous biological materials, including humans, animals, microorganisms and various plant varieties, has been, and is being carried out on a large scale. Polynucleotides identified using sequencing techniques may be partial or full-length genes, and may contain open reading frames, or portions of open reading frames, that encode polypeptides. Putative polypeptides may be determined based on polynucleotide sequences. The sequencing data relating to polynucleotides thus represents valuable and useful information.

[003] Polynucleotides may be analyzed for various degrees of novelty by comparing identified sequences to sequences published in various public domain databases, such as EMBL. Newly identified polynucleotides and putative polypeptides may also be compared to polynucleotides and polypeptides contained in public domain information to ascertain homology to known polynucleotides and polypeptides. In this way, the degree of similarity, identity, or homology of polynucleotides and polypeptides of unknown function may be determined relative to polynucleotides and polypeptides having known functions.

[004] Information relating to the sequences of isolated polynucleotides may be used in a variety of ways. Specified polynucleotides having a particular sequence may be isolated, or synthesized, for use in *in vivo* or *in vitro* experimentation as probes or primers. Alternatively, collections of sequences of isolated polynucleotides may be stored using magnetic or optical storage medium, and analyzed or manipulated using computer hardware and software, as well as other types of tools.

**TECHNICAL FIELD**

[005] The present invention is related generally to novel polynucleotides and novel polypeptides encoded thereby, their compositions, antibodies directed thereto, and other agonists or antagonists thereto. The polynucleotides and polypeptides are useful in

diagnostic, prophylactic, and therapeutic applications for a variety of diseases, disorders, syndromes, and conditions, as well as in discovering new diagnostics, prophylactics, and therapeutics for such diseases, disorders, syndromes, and conditions (hereinafter disorders). The present invention also relates to methods of modulating biological activities through the use of the novel polynucleotides and novel polypeptides of the invention and through the use of agonists and antagonists, such as antibodies, thereto.

[006] This application further relates to the field of polypeptides that are associated with regulating cell growth and differentiation, that are over-expressed in cancer, and/or that can be associated with proliferation or inhibition of cancer growth, including hematopoietic cancers such as leukemias, lymphomas, and solid cancers such as pancreatic cancer, tracheal cancer, and lung cancer, for example, adenocarcinomas and/or squamous cell carcinomas. These polypeptides may also be associated with other conditions, such as inflammatory, immune, and metabolic disorders, as well as microbial infections, including viral, bacterial, fungal, and parasitic disorders.

[007] This application further relates to modulators of biological activity that can specifically bind to these polynucleotides or polypeptides, or otherwise specifically modulate their activity. For example, they can directly or indirectly induce antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), endocytosis, apoptosis, or recruitment of other cells to effect cell activation, cell inactivation, cell growth or differentiation or inhibition thereof, and cell killing.

#### SUMMARY OF THE INVENTION

1. A first nucleic acid molecule comprising a polynucleotide sequence chosen from at least one polynucleotide sequence according to SEQ. ID. NOS. 1-187; SEQ. ID. NOS. 375-484, or a complement thereof, or from at least one polypeptide sequence that encodes SEQ. ID. NOS. 188-374.

2. The nucleic acid molecule of claim 1, wherein the nucleic acid molecule is a DNA or a RNA molecule.

3. An animal injected with the nucleic acid molecule of claim 1.

4. A double-stranded isolated nucleic acid molecule comprising the first nucleic acid molecule of claim 1 and its complement.

5. The nucleic acid molecule of claim 4, wherein the first polynucleotide sequence encodes a polypeptide chosen from a polypeptide comprising a signal peptide, a

mature polypeptide that lacks a signal peptide, a signal peptide, a biologically active fragment of a polypeptide, a polypeptide lacking a signal peptide cleavage site, a polypeptide consisting essentially of a N-terminal fragment that contains a Pfam domain, and a polypeptide consisting essentially of a C-terminal fragment that contains a Pfam domain.

6. A second nucleic acid molecule comprising a second polynucleotide sequence that is at least about 70%, or about 80%, or about 90%, or about 95% homologous to the first nucleic acid molecule of claim 1.

7. A second isolated nucleic acid molecule comprising a second polynucleotide sequence that hybridizes to the first polynucleotide sequence of claim 1 under high stringency conditions.

8. The second isolated nucleic acid molecule of claim 6, wherein the second polynucleotide sequence is complementary to the first polynucleotide sequence.

9. A vector comprising the nucleic acid molecule of claim 1 and a promoter that drives the expression of the nucleic acid molecule.

10. The vector of claim 9, wherein the promoter is chosen from one or more of a promoter that is naturally contiguous to the nucleic acid molecule, a promoter that is not naturally contiguous to the nucleic acid molecule, an inducible promoter, a conditionally active promoter, a constitutive promoter, and a tissue specific promoter.

11. A host cell transformed, transfected, transduced, or infected with the nucleic acid molecule of claim 1.

12. The host cell of claim 11, wherein the cell is chosen from one or more of a prokaryotic cell, a eucaryotic cell, a human cell, a mammalian cell, an insect cell, a fish cell, a plant cell, and a fungal cell.

13. A nucleic acid composition comprising a pharmaceutically acceptable carrier or a buffer and one or more compositions chosen from the nucleic acid molecule of claim 1, the nucleic acid molecule of claim 4, the vector of claim 9, and the host cell of claim 11.

14. A substantially purified polypeptide comprising a polypeptide sequence chosen from at least one amino acid sequence according to SEQ. ID. NOS. 188-374.

15. An animal injected with the polypeptide of claim 14.

16. The polypeptide of claim 14, wherein the polypeptide has a function chosen from an agonist, an antagonist, a ligand, and a receptor.

17. The polypeptide of claim 14, wherein the polypeptide is chosen from a polypeptide comprising a signal peptide, a mature polypeptide that lacks a signal peptide, a

signal peptide, a biologically active fragment of a polypeptide, a polypeptide lacking a signal peptide cleavage site, a biologically active fragment consisting essentially of an N-terminal fragment containing a Pfam domain, a biologically active fragment consisting essentially of a C-terminal fragment containing a Pfam domain, an extracellular fragment, a ligand binding fragment, and a receptor binding fragment.

18. A polypeptide composition comprising the polypeptide molecule of claim 14 and a pharmaceutically acceptable carrier or a buffer.

19. A cell culture medium comprising the polypeptide of claim 14.

20. The cell culture medium of claim 19, further comprising responder cells chosen from one or more T cells, B cells, NK cells, dendritic cells, macrophages, muscle cells, stem cells, epithelial skin cells, fat cells, blood cells, brain cells, bone marrow cells, endothelial cells, retinal cells, bone cells, kidney cells, pancreatic cells, liver cells, spleen cells, prostate cells, cervical cells, ovarian cells, breast cells, tracheal cells, lung cells, liver cells, soft tissue cells, colorectal cells, cells of the gastrointestinal tract, and cancer cells.

21. The cell culture medium of claim 20, wherein the responder cells proliferate in the medium.

22. The cell culture medium of claim 20, wherein the responder cells are inhibited in the medium.

23. A cell culture comprising transfected cells, wherein the transfected cells are transfected with the polynucleotide of claim 1.

24. The cell culture of claim 23, further comprising responder cells chosen from one or more T cells, B cells, NK cells, dendritic cells, macrophages, muscle cells, stem cells, epithelial skin cells, fat cells, blood cells, brain cells, bone marrow cells, endothelial cells, retinal cells, bone cells, kidney cells, pancreatic cells, liver cells, spleen cells, prostate cells, cervical cells, ovarian cells, breast cells, tracheal cells, lung cells, liver cells, soft tissue cells, colorectal cells, cells of the gastrointestinal tract, and cancer cells.

25. The cell culture of claim 23, wherein the responder cells proliferate in the cell culture.

26. The cell culture of claim 23, wherein the responder cells are inhibited in the cell culture.

27. A method of making a transformed, transfected, transduced, or infected host cell comprising:

(a) providing a composition comprising the vector of claim 9, and

(b) allowing a host cell to come into contact with the vector to form a transformed, transfected, transduced, or infected host cell.

28. A method of making a polypeptide comprising:

- (a) providing a nucleic acid molecule that comprises a polynucleotide sequence encoding the polypeptide of claim 14;
- (b) introducing the nucleic acid molecule into an expression system; and
- (c) allowing the polypeptide to be produced.

29. A method of making a polypeptide comprising:

- (a) providing a composition comprising the host cell of claim 11;
- (b) culturing the host cell to produce the polypeptide; and
- (c) allowing the polypeptide to be produced.

30. A diagnostic kit comprising a polynucleotide molecule, wherein the polynucleotide molecule comprises a sequence chosen from (a) at least 6, (b) at least 7, (c) at least 8, and (d) at least 9 contiguous nucleotides chosen from the nucleic acid molecule of claim 1.

31. A diagnostic kit comprising a polypeptide molecule, wherein the polypeptide molecule comprises an amino acid sequence or a biologically active fragment thereof, derived from the nucleic acid molecule of claim 1.

32. A genetically modified mouse comprising a deletion, substitution, or modification of a sequence chosen from SEQ. ID. NOS. 1-187; SEQ. ID. NOS. 375-484, wherein the deletion, substitution or modification prevents or reduces expression of said sequence and results in a mouse deficient in or completely lacking one or more gene products of a sequence chosen from SEQ. ID. NOS. 1-187; SEQ. ID. NOS. 375-484.

33. A method of determining the presence of the nucleic acid molecule of claim 1 or its complement comprising:

- (a) providing a complement to the nucleic acid molecule or providing a complement to the complement of the nucleic acid molecule;
- (b) allowing the molecules to interact; and
- (c) determining whether interaction has occurred.

34. A method of determining the presence of an antibody to the polypeptide of claim 14 in a sample, comprising:

- (a) providing the polypeptide;

(b) allowing the polypeptide to interact with any specific antibody in the sample;  
and

(c) determining whether interaction has occurred.

35. An antibody specifically recognizing, binding to, and/or modulating the biological activity of at least one polypeptide encoded by a nucleic acid molecule of claim 1, or a biologically active fragment thereof.

36. An antibody composition comprising the antibody of claim 35 and a pharmaceutically acceptable carrier.

37. The antibody of claim 35, wherein the antibody is chosen from one or more of a monoclonal antibody, a polyclonal antibody, a single chain antibody, an antibody comprising a backbone of a molecule with an Ig domain, a targeting antibody, a neutralizing antibody, a stabilizing antibody, an enhancing antibody, an antibody agonist, an antibody antagonist, an antibody that promotes endocytosis of a target antigen, a cytotoxic antibody, an antibody that mediates ADCC, a human antibody, a non-human primate antibody, a non-primate animal antibody, a rabbit antibody, a mouse antibody, a rat antibody, a sheep antibody, a goat antibody, a horse antibody, a porcine antibody, a cow antibody, a chicken antibody, a humanized antibody, a primatized antibody, and a chimeric antibody.

38. The antibody of claim 37, wherein the antibody is produced in a manner chosen from *in vivo* and *in vitro*.

39. The antibody of claim 37, wherein the antibody is produced in an organism chosen from a prokaryote and a eukaryote.

40. The antibody of claim 39, wherein the organism is chosen from a bacterial cell, a fungal cell, a plant cell, an insect cell, and a mammalian cell.

41. The antibody of claim 40, wherein the cell is chosen from a yeast cell, an *Aspergillus* cell, an SF9 cell, a High Five cell, a cereal plant cell, a tobacco cell, and a tomato cell.

42. The cytotoxic antibody of claim 37, further comprising one or more cytotoxic component chosen from a radioisotope, a microbial toxin, a plant toxin, and a chemical compound.

43. The cytotoxic antibody of claim 42, wherein the chemical compound is chosen from doxorubicin and cisplatin.

44. The antibody of claim 35, wherein the antibody has a function chosen from specifically inhibiting the binding of the polypeptide to a ligand, specifically inhibiting the

binding of the polypeptide to a substrate, specifically inhibiting the binding of the polypeptide as a ligand, and specifically inhibiting the binding of the polypeptide as a substrate.

45. A bacteriophage, wherein the antibody of claim 35, or a fragment thereof, is displayed on the bacteriophage.

46. A bacterial cell comprising the bacteriophage of claim 45.

47. A non-human animal injected with the antibody composition of claim 36.

48. A host cell that secretes the antibody of claim 35.

49. A method of making an antibody, comprising:

(a) introducing a polypeptide, polynucleotide encoding the polypeptide, or a biologically active fragment thereof into an animal in sufficient amount to elicit generation of antibodies specific to the polypeptide, wherein the polypeptide:

(i) is encoded by the nucleic acid molecule of claim 1; or

(ii) comprises the polypeptide sequence of claim 14; and

(b) recovering the antibodies therefrom.

50. The method of claim 49, further comprising after step (a), the step of isolating a spleen from the animal injected with the polypeptide or polynucleotide or a fragment thereof, and the step of recovering the antibodies from the spleen cells.

51. The method of claim 50, further comprising the step of making a hybridoma using cells from the spleen and selecting a hybridoma that secretes the antibodies.

52. The method of claim 50, further comprising making a polynucleotide library from the spleen cells, selecting a cDNA clone that produces the antibodies, and expressing the cDNA clone in an expression system to produce antibodies or fragments thereof.

53. A method of modulating biological activity comprising:

(a) providing the antibody of claim 35; and

(b) contacting the antibody with a first human or a non-human host cell thereby modulating the activity of a first human or non-human animal host cell, or a second host cell.

54. The method of claim 53, wherein the modulation of biological activity is chosen from enhancing cell activity directly, enhancing cell activity indirectly, inhibiting cell activity directly, and inhibiting cell activity indirectly.

55. The method of claim 53, wherein the step of contacting the antibody with a first human or non-human host cells results in recruitment of the second host cell.

56. The method of claim 53, wherein the first host cell is a cancer cell.

57. The method of claim 53, wherein the first or second host cell is chosen from a T cell, B cell, NK cell, dendritic cell, macrophage, muscle cell, stem cell, skin cell, fat cell, blood cell, brain cell, bone marrow cell, endothelial cell, retinal cell, bone cell, kidney cell, pancreatic cell, liver cell, spleen cell, prostate cell, cervical cell, ovarian cell, breast cell, tracheal cells, lung cell, liver cell, soft tissue cell, colorectal cell, and gastrointestinal tract cell.

58. A method of diagnosing a disease, disorder, syndrome, or condition chosen from cancer, proliferative, inflammatory, immune, metabolic, genetic, bacterial, and viral diseases, disorders, syndromes, or conditions in a patient, comprising:

- (a) providing the antibody of claim 35;
- (b) allowing the antibody to contact a patient sample; and
- (c) detecting specific binding between the antibody and an antigen in the sample to determine whether the subject has cancer, a proliferative, inflammatory, immune, metabolic, genetic, bacterial, or viral disease, disorder, syndrome, or condition.

59. A method of diagnosing a disease, disorder, syndrome, or condition chosen from cancer, proliferative, inflammatory, immune, bacterial, and viral diseases, disorders, syndromes, or conditions in a patient, comprising:

- (a) providing a polypeptide that specifically binds the antibody of claim 35;
- (b) allowing the polypeptide to contact a patient sample; and
- (c) detecting specific binding between the polypeptide and any interacting molecule in the sample to determine whether the subject has cancer, a proliferative, inflammatory, immune, bacterial, or viral disease, disorder, syndrome, or condition.

60. A method of identifying an agent that modulates the biological activity of a polypeptide comprising:

- (a) providing a polypeptide or an active fragment thereof, wherein the polypeptide comprises at least one amino acid sequence according to SEQ. ID. NOS. 188-374;
- (b) allowing at least one agent to contact the polypeptide; and
- (c) selecting an agent that binds the polypeptide or affects the biological activity of the polypeptide.



61. The method of claim 60, wherein the polypeptide is expressed on a cell surface.
62. A modulator composition comprising a modulator and a pharmaceutically acceptable carrier, wherein the modulator is obtainable by the method of claim 60.
63. The modulator composition of claim 62, wherein the modulator is an antibody.
64. A method of treating a disease, disorder, syndrome, or condition in a subject, comprising administering the composition of any one of claims 13, 18, and 36 to the subject.
65. The method of claim 64, wherein the composition is administered in a manner chosen from orally, parenterally, by implantation, by inhalation, intranasally, intravenously, intra-arterially, intracardiacally, subcutaneously, intraperitoneally, transdermally, intraventricularly, intracranially, and intrathecally.
66. The method of claim 64, wherein the disease, disorder, syndrome, or condition is chosen from cancer, a proliferative, inflammatory, immune, metabolic, genetic, bacterial, and viral disease, disorder, syndrome, or condition.
67. The method of claim 64, wherein the disease is cancer.
68. A method of treating a disease, disorder, syndrome, or condition chosen from cancer, proliferative, inflammatory, immune, metabolic, genetic, bacterial, and viral diseases, disorders, syndromes, or conditions in a subject, comprising:
- (a) providing an antibody composition that comprises a first antibody or fragment thereof that specifically binds to a first epitope of a first polypeptide or a biologically active fragment thereof, wherein the first polypeptide:
    - (i) is encoded by the nucleic acid molecule of claim 1; or
    - (ii) comprises the polypeptide of claim 14; and
  - (b) administering the antibody composition to the subject.
69. The method of claim 68, wherein the antibody composition further comprises a second antibody that binds specifically to or interferes with the activity of a second epitope of the first polypeptide or to a first epitope of a second polypeptide.
70. The method of claim 69, wherein the second polypeptide comprises the polypeptide of 14.
71. A kit comprising the antibody of claim 35 and instructions for its use.
72. A method of gene therapy, comprising:
- (a) providing a polynucleotide comprising a nucleic acid molecule encoding the antibody of claim 35; and

(b) administering the polynucleotide to a subject.

73. A method for prophylactic or therapeutic treatment of a subject, comprising:

(a) providing a vaccine; and

(b) administering the vaccine to the subject;

wherein the vaccine comprises a polynucleotide or a polypeptide chosen from at least one sequence according to SEQ. ID. NOS. 1 - 484 or a biologically active fragment thereof.

74. The method of claim 73, wherein the vaccine is a cancer vaccine, and the polypeptide is a cancer antigen.

75. A method of inhibiting transcription or translation of a first polynucleotide encoding a first polypeptide, comprising:

(a) providing a second polynucleotide that hybridizes to the first polynucleotide, wherein the first polynucleotide comprises a polynucleotide sequence chosen from:

(i) at least one polynucleotide sequence according to SEQ. ID. NOS. 1-187 or 375-484;

(ii) a polynucleotide encoding a polypeptide comprising an amino acid sequence chosen from at least one amino acid sequence according to SEQ. ID. NOS. 188-374; and

(iii) a polynucleotide encoding a fragment of a polypeptide comprising an amino acid sequence chosen from at least one amino acid sequence according to SEQ. ID. NOS. 188-374; and

(b) allowing the first polynucleotide to contact the second polynucleotide.

76. A method of treating a disease, disorder, syndrome, or condition comprising administering a modulator to a subject, wherein the modulator binds to a cell surface molecule that is over-expressed in the disease, disorder, syndrome, or condition, and is linked to the antibody of claim 35.

77. The method of claim 76, wherein the antibody is capable of initiating antibody-dependent cellular cytotoxicity.

78. The method of claim 76, wherein the disease, disorder, syndrome, or condition is cancer and the cell surface molecule is over-expressed in a cancer cell.

79. An isolated modified cell comprising at least one first heterologous nucleic acid molecule, wherein the first heterologous nucleic acid molecule comprises a first

polynucleotide sequence that encodes a first polypeptide, wherein the first polypeptide is chosen from the appendices and functions as a first therapeutic, diagnostic or prophylactic molecule for a disease, disorder, syndrome, or condition.

80. The cell of claim 79, wherein the disease, disorder, syndrome, or condition is chosen from cancer and other hyperproliferative diseases, CNS diseases, hematopoietic diseases, diabetes and other metabolic diseases, bone diseases, immune diseases, heart diseases, liver diseases, lung diseases, fertility-related diseases, kidney diseases, joint diseases, and related disorders, syndromes, or conditions.

81. The cell of claim 79, wherein the first polynucleotide sequence is chosen from a secreted molecule, an extracellular domain of a transmembrane molecule, a transmembrane molecule, and a receptor.

82. The cell of claim 79, wherein the first polynucleotide sequence is chosen from a growth factor, an anti-inflammatory factor, an anti-apoptotic factor, a vasodilator, an anti-coagulant, insulin, an anti-cancer agent, a signaling molecule, a biologically active fragment of the molecule, and a fusion protein comprising the molecule or biologically active fragment thereof.

83. The cell of claim 79, wherein the first polynucleotide sequence is chosen from an extracellular domain of a receptor, an immunoglobulin chain, a biologically active fragment of the molecule, and a fusion protein comprising the molecule or biologically active fragment thereof.

84. The cell of claim 79, wherein the first heterologous nucleic acid molecule encodes a fusion protein which is cleavable to release a biologically active molecule or biologically active fragment thereof.

85. The cell of claim 79, further comprising at least one second heterologous nucleic acid molecule that comprises at least one second polynucleotide sequence that encodes a second polypeptide.

86. The cell of claim 79, further comprising at least one second heterologous nucleic acid sequence encoding a second therapeutic molecule that functions in conjunction with the first therapeutic molecule.

87. The cell of claim 82, wherein the first therapeutic molecule is a growth factor chosen from a factor that stimulates cell proliferation, a factor that stimulates cell differentiation, a factor that promotes change in the metabolic state of a cell, and a factor that promotes change in the gene expression pattern of a cell.

88. The cell of claim 87, wherein the change in the metabolic state comprises activation, inactivation, or inhibition.
89. The cell of claim 79, wherein the first polypeptide is a growth factor.
90. The cell of claim 79, wherein the first polypeptide is an anti-inflammatory factor.
91. The isolated modified cell of claim 79, further comprising at least one third heterologous nucleic acid molecule that comprises a third polynucleotide sequence encoding a third polynucleotide.
92. The cell of claim 91, wherein the third polypeptide is a survival factor.
93. The cell of claim 92, wherein the survival factor is an anti-apoptotic factor.
94. The cell of claim 92, wherein the survival factor is Akt.
95. The cell of claim 92, wherein the survival factor is a reverse transcriptase.
96. The cell of claim 92, wherein the survival factor is telomerase.
97. The cell of claim 79, wherein the first heterologous nucleic acid sequence is under the regulatory control of a promoter.
98. The cell of claim 97, wherein the promoter is an inducible promoter.
99. The cell of claim 97, wherein the promoter is a constitutive promoter.
100. The cell of claim 97, wherein the promoter is a tissue-specific promoter.
101. The cell of claim 100, wherein the tissue-specific promoter enables expression of the first therapeutic molecule in a tissue chosen from liver, trachea, lung, heart, kidney, pancreas, brain, spinal cord, blood, bone marrow, joint, synovium, skin, GI tract, epithelial tissues, ovary, prostate, breast, spleen, bone, and cartilage.
102. The cell of claim 101, wherein the cell is differentiated into a tissue chosen from liver, trachea, lung, heart, kidney, pancreas, brain, spinal cord, blood, bone marrow, joint, synovium, skin, GI, epithelial tissues, ovary, prostate, breast, spleen, bone, and cartilage.
103. The cell of claim 97, wherein the promoter is heterologous to the first heterologous nucleic acid molecule.
104. The cell of claim 97, wherein the promoter is homologous to the first heterologous nucleic acid molecule.
105. A composition comprising the cell of claim 79 and a pharmaceutically acceptable carrier.

106. A kit comprising the composition of claim 106 and instructions for administration into an animal.

107. The kit of claim 106, wherein the animal is a human.

108. The cell of claim 87, wherein the second polynucleotide sequence is chosen from the appendices.

109. The cell of claim 108, wherein the first heterologous nucleic acid sequence encodes one member of a dimeric molecule and the second heterologous nucleic acid sequence encodes a second member of the dimeric molecule.

110. The cell of claim 109, wherein the dimeric molecule is a therapeutic molecule.

111. The cell of claim 109, wherein the dimeric molecule is an antibody.

112. The cell of claim 108, wherein the first heterologous nucleic acid sequence encodes an immunoglobulin heavy chain or a biologically active fragment thereof and the second heterologous nucleic acid sequence encodes an immunoglobulin light chain or a biologically active fragment thereof.

113. The cell of claim 111, wherein the antibody is an anti-inflammation antibody.

114. The cell of claim 111, wherein the antibody is an anti-cancer antibody.

115. A composition comprising the cell of claim 87 and a pharmaceutically acceptable carrier.

116. A kit comprising the composition of claim 115 and instructions for administration into an animal.

117. The kit of claim 116, wherein the animal is a human.

118. The isolated modified cell of claim 111, wherein the antibody is chosen from a monoclonal antibody, a single chain antibody, and a biologically active fragment thereof.

119. A method for treatment of a disease, disorder, syndrome, or condition in a subject comprising the steps of:

- (a) providing a composition comprising a plurality of isolated modified cells, wherein each modified cell comprises at least one first heterologous nucleic acid molecule, wherein the first heterologous nucleic acid molecule comprises a first polynucleotide sequence that encodes a first polypeptide, wherein the first polypeptide is chosen from the appendices; and
- (b) administering the composition to the subject.

120. The method of claim 119, wherein the disease, disorder, syndrome, or condition is a hyperproliferative disease, disorder, syndrome, or condition.

121. The method of claim 120, wherein the hyperproliferative disease, disorder, syndrome, or condition is chosen from cancer, psoriasis, and ulcerative colitis.

122. The method of claim 119, wherein the disease, disorder, syndrome, or condition is inflammation and the first polypeptide is an anti-inflammatory molecule.

123. The method of claim 119, wherein the disease, disorder, syndrome, or condition is ischemic heart disease and the first polypeptide is a vasodilator.

124. The method of claim 119, wherein the disease, disorder, syndrome, or condition is thrombosis and the first therapeutic molecule is an anti-coagulant.

125. The method of claim 119, wherein the disease, disorder, syndrome, or condition is immunological, and the first polypeptide is immunosuppressive.

126. The method of claim 119, wherein the disease, disorder, syndrome, or condition is immunological, and the first polypeptide enhances the immune response of the subject.

127. A method for treatment of a disease, disorder, syndrome, or condition in a subject comprising the steps of:

(a) providing a composition comprising a plurality of isolated modified cells of claim 87; and

(b) administering the composition to the subject.

128. The method of claim 127, wherein the disease, disorder, syndrome, or condition is a hyperproliferative disease, disorder, syndrome, or condition.

129. The method of claim 128, wherein the hyperproliferative disease, disorder, syndrome, or condition is chosen from among cancer, psoriasis, and ulcerative colitis.

130. The method of claim 129, wherein the hyperproliferative disease, disorder, syndrome, or condition is psoriasis.

131. A non-human animal deficient in a polypeptide chosen from SEQ. ID. NOS. 188 to 374.

132. The non-human animal of claim 131 in which a polynucleotide encoding the polypeptide is functionally defective or missing.

133. The non-human animal of claim 131, wherein the animal is a mouse.

134. The non-human animal of claim 131, wherein the non-human animal suffers from a disease, disorder, syndrome, or condition chosen from abnormal growth, hormonal imbalance, early aging, abnormal bone formation, immune disorder, cancer, diabetes, skin disease, and CNS disease.

135. Isolated tissues derived from the non-human animal of claim 131.

136. One or more cells derived from the non-human animal of claim 131.

137. The cell(s) of claim 136, wherein the cell(s) are chosen from stem cells, bone marrow cells, liver cells, pancreatic cells, skin cells, hematopoietic cells, kidney cells, heart cells, epithelial cells, neuronal cells, and lung cells.

138. The cell(s) of claim 136, wherein the cells are chosen from precursor cells, progenitor cells, and differentiated cells of the hematopoietic lineage.

139. The cell(s) of claim 136, wherein the cells are bone marrow cells, and the bone marrow cells are chosen from stromal cells and hematopoietic cells.

140. A non-human animal that over-expresses a polypeptide chosen from SEQ. ID. NOS. 188 to 374.

141. The non-human animal of claim 140, wherein the polypeptide is encoded by a nucleic acid molecule comprising an exogenous DNA sequence.

142. The non-human animal of claim 140, wherein the animal is a mouse.

143. The non-human animal of claim 140, wherein the non-human animal suffers from a disease, disorder, syndrome, or condition chosen from abnormal growth, hormonal imbalance, early aging, abnormal bone formation, immune disorder disease, cancer, diabetes, skin disease, and CNS disease.

144. One or more isolated tissues derived from the non-human animal of claim 140.

145. One or more cells derived from the non-human animal of claim 140.

146. The cell(s) of claim 145, wherein the cells are chosen from stem cells, bone marrow cells, liver cells, pancreatic cells, skin cells, hematopoietic cells, kidney cells, heart cells, epithelial cells, neuronal cells, and lung cells.

147. The cell(s) of claim 145, wherein the cells are chosen from precursor cells, progenitor cells, mesenchymal cells, stem cells, and differentiated cells of the hematopoietic lineage.

148. The cell(s) of claim 145, wherein the cells are bone marrow cells, and the bone marrow cells are chosen from stromal cells and hematopoietic cells.

#### **DISCLOSURE OF THE INVENTION**

[008] The present invention features an isolated polynucleotide that encodes a polypeptide. In some embodiments, the polypeptide has at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least

about 97%, at least about 98%, or at least about 99% amino acid sequence identity with an amino acid sequence derived from a polynucleotide sequence chosen from at least one nucleotide sequence according to SEQ. ID. NOS. 1-187 and 375-484. In some embodiments, the polypeptide has an amino acid sequence chosen from at least one amino acid sequence according to SEQ. ID. NOS. 188-374. In many embodiments, the polypeptide has at least one activity associated with the naturally occurring encoded polypeptide.

[009] In some embodiments, the polypeptide includes a signal peptide. In alternative embodiments, the polypeptide comprises a mature form of a protein, from which the signal peptide has been cleaved. In other embodiments, the polypeptide is a signal peptide. In a further aspect, the invention provides fragments of a polypeptide chosen from at least one amino acid sequence according to SEQ. ID. NOS. 188-374, where each fragment is an extracellular fragment of the polypeptide, or an extracellular fragment of the polypeptide minus the signal peptide. The invention provides an N-terminal fragment containing a Pfam domain and a C-terminal fragment containing a Pfam domain and either or both may be biologically active.

[010] In yet other embodiments, the polypeptides function as secreted proteins. In yet further embodiments, the polypeptides function as single-transmembrane proteins. In yet further embodiments, the polypeptides function as multiple-transmembrane proteins. In yet further embodiments, the polypeptides function as kinases, *e.g.*, protein kinases.

[011] The present invention features an isolated polynucleotide that hybridizes under stringent hybridization conditions to a coding region of at least one nucleotide sequence shown in SEQ. ID. NOS. 1-187, 375-484, or a complement thereof.

[012] The present invention features an isolated polynucleotide that shares at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 97%, at least about 98%, at least about 99% nucleotide sequence identity with a nucleotide sequence of the coding region of at least one sequence shown in SEQ. ID. NOS. 1-187, 375-484, or a complement thereof. In some embodiments, a subject polynucleotide has the nucleotide sequence shown in at least one of SEQ. ID. NOS. 1-187, 375-484, or a coding region thereof.

[013] The present invention also features a vector, *e.g.*, a recombinant vector, that includes a subject polynucleotide, and a promoter that drives its expression. This vector can transform a host cell, and the present invention further features such host cells, *e.g.*, isolated *in*



*vitro* host cells, and *in vivo* host cells, that comprise a polynucleotide of the invention, or a recombinant vector of the invention.

[014] The present invention further features a library of polynucleotides, wherein at least one of the polynucleotides comprises the sequence information of a polynucleotide of the invention. In specific embodiments, the library is provided on a nucleic acid array. In some embodiments, the library is provided in computer-readable format.

[015] The present invention features a pair of isolated nucleic acid molecules, each from about 10 to about 200 nucleotides in length. The first nucleic acid molecule of the pair comprises a sequence of at least 10 contiguous nucleotides having 100% sequence identity to at least one nucleic acid sequence shown in SEQ. ID. NOS. 1-187 and 375-484. The second nucleic acid molecule of the pair comprises a sequence of at least 10 contiguous nucleotides having 100% sequence identity to the reverse complement of at least one nucleic acid sequence shown in SEQ. ID. NOS. 1-187 and 375-484. The sequence of said second nucleic acid molecule is located 3' of the nucleic acid sequence of the first nucleic acid molecule shown in SEQ. ID. NOS. 1-187 and 375-484. The pair of isolated nucleic acid molecules are useful in a polymerase chain reaction or in any other method known in the art to amplify a nucleic acid that has sequence identity to the sequences shown in SEQ. ID. NOS. 1-187 and 375-484, particularly when cDNA is used as a template.

[016] The invention features a method of determining the presence of a polynucleotide substantially identical to a polynucleotide sequence shown in the appendices, or a complement of such a nucleotide by providing its complement, allowing the polynucleotides to interact, and determining whether such interaction has occurred.

[017] The invention further features methods of regulating the expression of the subject polynucleotides and encoded polypeptides. The invention provides a method of inhibiting transcription or translation of a first polynucleotide encoding a first polypeptide of the invention by providing a second polynucleotide that hybridizes to the first polynucleotide, and allowing the first polynucleotide to contact and bind to the second polynucleotide. The second polynucleotide can be chosen from an antisense molecule, a ribozyme, and an interfering RNA (RNAi) molecule.

[018] The present invention further features an isolated polypeptide, *e.g.*, an isolated polypeptide encoded by a polynucleotide, and biologically active fragments of such polypeptide. In some embodiments, the polypeptide is a fusion protein. In some embodiments, the polypeptide has one or more amino acid substitutions, and/or insertions

and/or deletions, compared with at least one sequence shown in SEQ. ID. NOS. 188-374. In some embodiments, the polypeptide has an amino acid sequence derived from at least one nucleotide sequence shown in SEQ. ID. NOS. 1-187 and 375-484. In some embodiments, the polypeptide has an amino acid sequence substantially identical to at least one sequence shown in SEQ. ID. NOS. 188-374.

[019] The invention also provides a method of making a polypeptide of the invention by providing a nucleic acid molecule that comprises a polynucleotide sequence encoding a polypeptide of the invention, introducing the nucleic acid molecule into an expression system, and allowing the polypeptide to be produced.

[020] In some embodiments, the method involves *in vitro* cell-free transcription and/or translation. For example, the expression system can comprise a cell-free expression system, such as an *E. coli* system, a wheat germ extract system, a rabbit reticulocyte system, or a frog oocyte system.

[021] In certain other embodiments, the expression system can comprise a prokaryotic or eukaryotic cell, for example, a bacterial cell expression system, a fungal cell expression system, such as yeast or *Aspergillus*, a plant cell expression system, *e.g.*, a cereal plant, a tobacco plant, a tomato plant, or other edible plant, an insect cell expression system, such as SF9 of High Five cells, an amphibian cell expression system, a reptile cell expression system, a crustacean cell expression system, an avian cell expression system, a fish cell expression system, or a mammalian cell expression system, such as one using Chinese Hamster Ovary (CHO) cells. In some embodiments, the method involves culturing a subject host cell under conditions such that the subject polypeptide is produced by the host cells; and recovering the subject polypeptide from the culture, *e.g.*, from within the host cells, or from the culture medium. In further embodiments, the polypeptide can be produced *in vivo* in a multicellular animal or plant, comprising a polynucleotide encoding the subject polypeptide.

[022] The present invention further features a non-human animal injected with at least one polynucleotide comprising at least one nucleotide sequence chosen from SEQ. ID. NOS. 1-187 and 375-484, and/or at least one polypeptide comprising at least one amino acid sequence chosen from SEQ. ID. NOS. 188-374.

[023] The present invention further features an antibody that specifically recognizes, binds to, interferes with, or modulates the biological activity of a subject polypeptide or a fragment thereof. The present invention features an antibody that specifically inhibits

binding of a polypeptide to its ligand or substrate. It also features an antibody that specifically inhibits binding of a polypeptide as a substrate to another molecule.

[024] Another aspect of the present invention features a library of antibodies or fragments thereof, wherein at least one antibody or fragment thereof specifically binds to at least a portion of a polypeptide comprising an amino acid sequence according to SEQ. ID. NOS. 188-374 , and/or wherein at least one antibody or fragment thereof interferes with at least one activity of such polypeptide or fragment thereof. In certain embodiments, the antibody library comprises at least one antibody or fragment thereof that specifically inhibits binding of a subject polypeptide to its ligand or substrate, or that specifically inhibits binding of a subject polypeptide as a substrate to another molecule. The present invention also features corresponding polynucleotide libraries comprising at least one polynucleotide sequence that encodes an antibody or antibody fragment of the invention. In specific embodiments, the library is provided on a nucleic acid array or in computer-readable format.

[025] An antibody of the present invention may comprise a monoclonal antibody, polyclonal antibody, single chain antibody, intrabody, and active fragments of any of these. The active fragments include variable regions from either heavy chains or light chains. The antibody can comprise the backbone of a molecule with an immunoglobulin domain, *e.g.*, a fibronectin backbone, a T-cell receptor backbone, or a CTLA4 backbone.

[026] The present invention further features a targeting antibody, a neutralizing antibody, a stabilizing antibody, an enhancing antibody, an antibody agonist, an antibody antagonist, an antibody that promotes cellular endocytosis of a target antigen, a cytotoxic antibody, and an antibody that mediates antibody dependent cellular cytotoxicity (ADCC). The antibody that mediates ADCC can have a cytotoxic component, *e.g.*, a radioisotope, a radioactive molecule, a microbial toxin, a plant toxin, a chemotherapeutic agent, or a chemical substance, such as doxorubicin or cisplatin. The invention also features an inhibitory antibody, functioning to specifically inhibit the binding of a cognate polypeptide to its ligand or its substrate, or to specifically inhibit the binding of a cognate peptide as the substrate of another molecule.

[027] The antibodies of the present invention also encompass a human antibody, a non-human primate antibody, a monkey antibody, a non-primate animal antibody, *e.g.*, a rodent antibody, rat antibody, a mouse antibody, a hamster antibody, a guinea pig antibody, a chicken antibody, a cattle antibody, a sheep antibody, a goat antibody, a horse antibody,

porcine antibody, a cow antibody, a rabbit antibody, a cat antibody, or a dog antibody. It also features a humanized antibody, a primatized antibody, and a chimeric antibody.

[028] The antibodies of the invention can be produced *in vitro* or *in vivo*. For example, the present invention features an antibody produced in a cell-free expression system, a prokaryote expression system or a eukaryote expression system, as described herein.

[029] The invention further provides a host cell that can produce an antibody of the invention or a fragment thereof. The antibody may also be secreted by the cell. The host cell can be a hybridoma, or a prokaryotic or eukaryotic cell. The invention also provides a bacteriophage or other virus particle comprising an antibody of the invention, or a fragment thereof. The bacteriophage or other virus particle may display the antibody or fragment thereof on its surface, and the bacteriophage itself may exist within a bacterial cell. The antibody may also comprise a fusion protein with a viral or bacteriophage protein.

[030] The invention further provides transgenic multicellular organisms, *e.g.*, plants or non-human animals, as well as tissues or organs, comprising a polynucleotide sequence encoding a subject antibody or fragment thereof. The organism, tissues, or organs will generally comprise cells producing an antibody of the invention, or a fragment thereof.

[031] In another aspect, the present invention features a method of making an antibody by immunizing a host animal. In this method, a polypeptide or a fragment thereof, a polynucleotide encoding a polypeptide, or a polynucleotide encoding a fragment thereof, is introduced into an animal in a sufficient amount to elicit the generation of antibodies specific to the polypeptide or fragment thereof, and the resulting antibodies are recovered from the animal. The polypeptide can be encoded by a nucleic acid molecule comprising a nucleotide sequence chosen from at least one polynucleotide sequence according to SEQ. ID. NOS. 1-187 and 375-484. For example, the polypeptide may comprise at least one amino acid sequence chosen from SEQ. ID. NOS. 188-374.

[032] The invention thus also provides a non-human animal comprising an antibody of the invention. The animal can be a non-human primate, (*e.g.*, a monkey) a rodent (*e.g.*, a rat, a mouse, a hamster, a guinea pig), a chicken, cattle (*e.g.*, a cow), a sheep, a goat, a horse, a pig, a rabbit, a cat, or a dog.

[033] The present invention also features a method of making an antibody by isolating a spleen from an animal injected with a polypeptide or a fragment thereof, a polynucleotide encoding a polypeptide, or a polynucleotide encoding a fragment thereof, and

recovering antibodies from the spleen cells. Hybridomas can be made from the spleen cells, and hybridomas secreting specific antibodies can be selected.

[034] The present invention further features a method of making a polynucleotide library from spleen cells, and selecting a cDNA clone that produces specific antibodies, or fragments thereof. The cDNA clone or a fragment thereof can be expressed in an expression system that allows production of the antibody or a fragment thereof, as provided herein.

[035] The invention also provides a method for determining the presence or measuring the level of a polypeptide that specifically binds to an antibody of the invention. This method involves allowing the antibody to interact with a sample, and determining whether interaction between the antibody and any polypeptide in the sample has occurred. Antibodies that specifically bind to at least one subject polypeptide are useful in diagnostic assays, *e.g.*, to detect the presence of a subject polypeptide. Similarly, the invention features a method of determining the presence of an antibody to a polypeptide of the invention, by providing the polypeptide, allowing the antibody and the polypeptide to interact, and determining whether interaction has occurred.

[036] The present invention further features a method of identifying an agent that modulates the level of a subject polypeptide (or an mRNA encoding a subject polypeptide) in a cell. The method generally involves contacting a cell (*e.g.*, a eukaryotic cell) that produces the subject polypeptide with a test agent; and determining the effect, if any, of the test agent on the level of the polypeptide in the cell.

[037] The present invention further features a method of identifying an agent that modulates biological activity of a subject polypeptide. The methods generally involve contacting a subject polypeptide with a test agent; and determining the effect, if any, of the test agent on the activity of the polypeptide. In certain embodiments, the polypeptide is expressed on a cell surface. In certain embodiments, the agent or modulator is an antibody, for example, where an antibody binds to the polypeptide or affects its biological activity.

[038] The present invention further features biologically active agents (or modulators) identified using a method of the invention.

[039] The present invention also features a method of modulating biological activity using an agent selectable by the above methods. Briefly, the method of modulating biological activity comprises contacting the agent with a first human or a non-human host cell, thereby modulating the activity of the first host cell or a second host cell. In one example, contacting the agent with the first human or non-human host cell results in the

recruitment of a second host cell. The agent may be an antibody or antibody fragment of the invention.

[040] The modulation can comprise directly enhancing cell activity, indirectly enhancing cell activity, directly inhibiting cell activity, or indirectly inhibiting cell activity. The cell activity that is modulated can include transcription, translation, cell cycle control, signal transduction, intracellular trafficking, cell adhesion, cell mobility, proteolysis, ion transport, water transport, DNA repair, hydrolysis, lipase activity, polymerization using an RNA template or a DNA template, and nuclease activity. The modulation can result in cell death or apoptosis, or inhibition of cell death or apoptosis, as well as cell growth, cell proliferation, or cell survival, or inhibition of cell growth, cell proliferation, or cell survival; as well as mucosal preservation, inhibition of eicosanoid synthesis, or resistance to infection by viruses.

[041] Either the first or the second host cell can be a human or a non-human host cell. Either the first or the second host cell can be an immune cell, *e.g.*, a T cell, B cell, NK cell, dendritic cell, macrophage, muscle cell, stem cell, skin cell, fat cell, blood cell, brain cell, bone marrow cell, endothelial cell, retinal cell, bone cell, kidney cell, pancreatic cell, liver cell, spleen cell, prostate cell, cervical cell, ovarian cell, breast cell, tracheal cell, lung cell, liver cell, soft tissue cell, colorectal cell, other cell of the gastrointestinal tract, or a cancer cell.

[042] The invention also provides a method of diagnosing cancer, proliferative, inflammatory, immune, viral, bacterial, or metabolic disorder in a patient, by allowing an antibody specific for a polypeptide of the invention to contact a patient sample, and detecting specific binding between the antibody and any antigen in the sample to determine whether the subject has cancer, proliferative, inflammatory, immune, viral, bacterial, or metabolic disorder.

[043] The invention further provides a method of diagnosing cancer, proliferative, inflammatory, immune, viral, bacterial, or metabolic disorder in a patient, by allowing a polypeptide of the invention to contact a patient sample, and detecting specific binding between the polypeptide and any interacting molecule in the sample to determine whether the subject has cancer, proliferative, inflammatory, immune, viral, bacterial, or metabolic disorder.

[044] The invention also features a method of providing a polynucleotide, a polypeptide, or an agent of the invention, such as an antibody, to a subject by oral, buccal,

nasal, rectal, intraperitoneal, intradermal, transdermal, intratracheal, intrathecal, or parenteral administration, or otherwise by implantation or inhalation. For example, the polynucleotide, polypeptide or agent can be administered intranasally, intravenously, intra-arterially, intracardiacally, subcutaneously, intraperitoneally, transdermally, intraventricularly, or intracranially. The invention also provides a method for formulating a polynucleotide, polypeptide, or modulator composition, such as an antibody composition, for delivery by any of the routes of administration provided above, for example, for treatment of disorders. For example, the parenteral delivery can be via inhalation or implantation. The parenteral delivery can also be oral, intranasal, intraventricular, or intracranial.

[045] The present invention also features a pharmaceutical composition comprising a polynucleotide, polypeptide, or modulator of the invention and a carrier. The carrier can be a pharmaceutically acceptable carrier. The modulator can be obtainable by any methods of the invention, for example, the modulator can be an antibody or a fragment thereof. Further, oral formulations, preparations for injection, aerosol formulations, and suppositories can be prepared, each comprising the polynucleotide, polypeptide, or modulator composition. Further, nucleic acid compositions comprising polynucleotide sequences encoding the subject antibodies, or fragments thereof, can be prepared for administration to a subject.

[046] The invention also features a non-human animal injected with the polynucleotide, polypeptide, or modulator composition, for example the antibody composition. Again, the animal can be a non-human primate, (*e.g.*, a monkey) a rodent (*e.g.*, a rat, a mouse, a hamster, a guinea pig), a chicken, cattle (*e.g.*, a cow), a sheep, a goat, a horse, a pig, a rabbit, a cat, or a dog.

[047] In another aspect, the invention provides a method of treating a disorder in a subject needing or desiring such treatment, comprising administering a polynucleotide, polypeptide, or modulator of the invention to the subject. The subject can be a human or a non-human animal. The disorder can be cancer, proliferative, inflammatory, immune, metabolic, ulcerative, bacterial, or viral disorders.

[048] For example, the method of treatment may comprise administering an antibody composition with a first antibody that specifically binds to a first epitope of a first polypeptide or a fragment thereof, or that interferes with at least one activity of the first polypeptide or a fragment thereof, wherein the first polypeptide is encoded by a nucleic acid molecule comprising a nucleotide sequence chosen from SEQ. ID. NOS. 1-187 and 375-484, or any nucleic acid of the present invention. For example, the first polypeptide may comprise

an amino acid sequence chosen from SEQ. ID. NOS. 188-374. In certain embodiments, this method further comprises using a second antibody that binds specifically to or interferes with the activity of a second epitope of the first polypeptide or to a first epitope of a second polypeptide. The second polypeptide can be encoded by a nucleic acid molecule comprising a nucleotide sequence chosen from SEQ. ID. NOS. 1-187 and 375-484, or any nucleic acid of the present invention. For example, the second polypeptide may comprise an amino acid sequence chosen from SEQ. ID. NOS. 188-374. In certain embodiments, the antibody binds, or interferes with the activity of, at least one polypeptide fragment, wherein the fragment is an extracellular fragment of the polypeptide, or an extracellular fragment of the polypeptide minus the signal peptide, for the treatment, for example, of proliferative disorders, such as cancer.

[049] In other embodiments, the modulator may bind to a cell surface molecule that is over-expressed in the disorder. Further the modulator may be linked to an antibody of the invention. The antibody can be capable of initiating antibody dependent cell cytotoxicity, *e.g.*, where the antibody is in turn coupled to cytotoxic agents. This method is applicable when the disorder is cancer, another proliferative disorder, inflammatory, immune, bacterial, viral, or metabolic disorder, and the cell surface molecule is over-expressed in a cancer cell, diseased cell or virus-infected cell.

[050] The invention also provides a method for prophylactic or therapeutic treatment of a subject needing or desiring such treatment by providing a vaccine, that can be administered to the subject. The vaccine may comprise one or more of a polynucleotide, polypeptide, or modulator of the invention, for example an antibody vaccine composition, a polypeptide vaccine composition, or a polynucleotide vaccine composition, useful for treating cancer, proliferative, inflammatory, immune, metabolic, bacterial, or viral disorders.

[051] For example, the vaccine can be a cancer vaccine, and the polypeptide can concomitantly be a cancer antigen. The vaccine may be an anti-inflammatory vaccine, and the polypeptide can concomitantly be an inflammation-related antigen. The vaccine may be a viral vaccine, and the polypeptide can concomitantly be a viral antigen. In some embodiments, the vaccine comprises a polypeptide fragment, comprising at least one extracellular fragment of a polypeptide of the invention, and/or at least one extracellular fragment of a polypeptide of the invention minus the signal peptide, for the treatment, for example, of proliferative disorders, such as cancer. In certain embodiments, the vaccine comprises a polynucleotide encoding one or more such fragments, administered for the



treatment, for example, of proliferative disorders, such as cancer. Further, the vaccine can be administered with or without an adjuvant.

[052] In another aspect, the invention provides a method for gene therapy by providing a polynucleotide comprising a nucleic acid molecule encoding a polypeptide, such as an antibody of the invention, and administering the polynucleotide to a subject needing or desiring such treatment.

[053] The invention further provides a kit comprising one or more of a polynucleotide, polypeptide, or modulator composition, such as an antibody composition, which may include instructions for its use. Such kits are useful in diagnostic applications, for example, to detect the presence and/or level of a polypeptide in a biological sample by specific antibody interaction.

[054] Additional objects and advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice of the invention. The objects and advantages of the invention will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. Moreover, advantages described in the body of the specification, if not included in the claims, are not per se limitations to the claimed invention.

#### **Brief Description of the Tables and Appendices**

[055] Each sequence shown in Tables 1-3 is identified by an internal reference number (FP ID). Table 1 lists the sequences of the invention. Each is identified by its FP ID number, a SEQ. ID. NO. corresponding to the nucleotide coding sequence (SEQ. ID. NO. (N1), a SEQ. ID. NO. corresponding to the encoded polypeptide sequence (SEQ. ID. NO. (P1), and a SEQ. ID. NO. corresponding to the entire nucleotide sequence (SEQ. ID. NO. (N0). Each is also identified by a Clone ID designation that lists each novel clone of the invention.

[056] Table 2 describes structural characteristics of the polypeptides of the invention encoded by these novel clones. In addition to listing the FP ID and Clone ID, it specifies the predicted number of amino acid residues in the polypeptide (Predicted Protein Length). Table 2 also specifies the result of an algorithm that predicts whether a sequence is secreted (Tree Vote). This algorithm is constructed on the basis of a number of attributes that include hydrophobicity, two-dimensional structure, prediction of signal sequence cleavage site, and other parameters, and predicts whether the listed sequences are secreted polypeptides or nucleotides related to secreted polypeptides. Table 2 also specifies the positions of the amino

acid residues that comprise signal peptides (Signal Peptide Coords.), as well as the coordinates of the amino acid residues comprising the protein after the signal peptide sequence has been cleaved to produce a mature protein (Mature Protein Coords.). Additionally, Table 2 specifies the coordinates of an alternate form of a mature protein. In instances where the mature protein start residue overlaps the signal peptide end residue, some of the amino acid residues may be cleaved off such that the mature protein does not start at the next amino acid residue from the signal peptides, resulting in the alternative mature protein coordinate (Alternative Mature Protein Coords.). Table 2 specifies the number of Transmembrane domains of each of the polypeptides of the invention (TM). It specifies the positions of the amino acid residues that comprise the transmembrane domains (TM Coords.) and the positions of the amino acids that do not pass through the membrane (Non-TM Coords.). Finally, Table 2 specifies protein family (Pfam) classifications for some of these novel human cDNA clones.

[057] Table 3 describes the characteristics of the protein in the public National Center for Information Biotechnology (NCBI) database displaying the greatest degree of similarity to polypeptides encoded by each novel human cDNA clone of the invention. The NCBI protein with the greatest homology to each Clone ID is described by its NCBI accession number (Top Hit Accession ID), and by the NCBI's annotation of that sequence (Top Hit Annotation). The percent identity of the Five Prime protein with its corresponding NCBI protein is listed (Top Hit % ID). Table 3 also describes the characteristics of the human protein in the NCBI database with the greatest degree of similarity to polypeptides encoded by each novel human cDNA clone of the invention. The corresponding NCBI protein is described by its NCBI accession number (Top Human Hit Accession ID) and by the NCBI's annotation of that sequence (Top Human Hit Annotation). Finally, the percent identity of the Five Prime protein with the NCBI protein is listed (Top Human Hit % ID).

[058] Appendix A provides nucleotide sequences of the encoding regions of the subject nucleic acids. Appendix B provides amino acid sequences of the subject polypeptides, which correspond to the encoding sequences in Appendix A. Appendix C provides nucleotide sequences of the DNA sequences used to find the encoding sequences of the subject nucleic acids. The appendices are provided as a paper copy.

## **Definitions**

[059] "Related sequences" include nucleotide and amino acid sequences that are involved in the function of their referent. For example, "receptor-related sequences" include

all sequences that are involved in receptor function. This includes, but is not limited to, sequences that are involved in receptor synthesis, receptor regulation, receptor effector function, and receptor degradation. "Related sequences" also encompass complementary nucleic acid sequences, and biologically active fragments of nucleic acid and amino acid sequences.

[060] The terms "polynucleotide," "nucleotide," "nucleic acid," "polynucleic molecule," "nucleotide molecule," "nucleic acid molecule," "nucleic acid sequence," "polynucleotide sequence," and "nucleotide sequence" are used interchangeably herein to refer to polymeric forms of nucleotides of any length. The polynucleotides can contain deoxyribonucleotides, ribonucleotides, and/or their analogs or derivatives. For example, nucleic acids can be naturally occurring DNA or RNA, or can be synthetic analogs, as known in the art. The terms also encompass genomic DNA, genes, gene fragments, exons, introns, regulatory sequences or regulatory elements (such as promoters, enhancers, initiation and termination regions, other control regions, expression regulatory factors, and expression controls), DNA comprising one or more single-nucleotide polymorphisms (SNPs), allelic variants, isolated DNA of any sequence, and cDNA. The terms also encompass mRNA, tRNA, rRNA, ribozymes, splice variants, antisense RNA, antisense conjugates, RNAi, and isolated RNA of any sequence. The terms also encompass recombinant polynucleotides, heterologous polynucleotides, branched polynucleotides, labeled polynucleotides, hybrid DNA/RNA, polynucleotide constructs, vectors comprising the subject nucleic acids, nucleic acid probes, primers, and primer pairs. The polynucleotides can comprise modified nucleic acid molecules, with alterations in the backbone, sugars, or heterocyclic bases, such as methylated nucleic acid molecules, peptide nucleic acids, and nucleic acid molecule analogs, which may be suitable as, for example, probes if they demonstrate superior stability and/or binding affinity under assay conditions. Analogs of purines and pyrimidines, including radiolabeled and fluorescent analogs, are known in the art. The polynucleotides can have any three-dimensional structure, and can perform any function, known or as yet unknown. The terms also encompass single-stranded, double-stranded and triple helical molecules that are either DNA, RNA, or hybrid DNA/RNA and that may encode a full-length gene or a biologically active fragment thereof. Biologically active fragments of polynucleotides can encode the polypeptides herein, as well as anti-sense and RNAi molecules. Thus, the full length polynucleotides herein may be treated with enzymes, such as Dicer, to generate a library of short RNAi fragments which are within the scope of the present invention.

[061] The novel polynucleotides herein include those shown in the Tables, SEQ. ID. NOS. 1-187 and 375-484, as well as those that encode the polypeptides of SEQ. ID. NOS. 188-374, and biologically active fragments thereof. The polynucleotides also include modified, labeled, and degenerate variants of the nucleic acid sequences, as well as nucleic acid sequences that are substantially similar or homologous to nucleic acids encoding the subject proteins.

[062] A "biologically active" entity, or an entity having "biological activity," is one having structural, regulatory, or biochemical functions of a naturally occurring molecule or any function related to or associated with a metabolic or physiological process. Biologically active polynucleotide fragments are those exhibiting activity similar, but not necessarily identical, to an activity of a polynucleotide of the present invention. The biological activity can include an improved desired activity, or a decreased undesirable activity. For example, an entity demonstrates biological activity when it participates in a molecular interaction with another molecule, or when it has therapeutic value in alleviating a disease condition, or when it has prophylactic value in inducing an immune response to the molecule, or when it has diagnostic value in determining the presence of the molecule, such as a biologically active fragment of a polynucleotide that can be detected as unique for the polynucleotide molecule, or that can be used as a primer in PCR.

[063] The term "degenerate variant" of a nucleic acid sequence refers to all nucleic acid sequences that can be directly translated, according to the standard genetic code, to provide an amino acid sequence identical to that translated from a reference nucleic acid sequence.

[064] The term "gene" or "genomic sequence" as used herein is an open reading frame encoding specific proteins and polypeptides, for example, an mRNA, cDNA, or genomic DNA, and also may or may not include intervening introns, or adjacent 5' and 3' non-coding nucleotide sequences involved in the regulation of expression up to about 20 kb beyond the coding region, and possibly further in either direction. A gene can be introduced into an appropriate vector for extrachromosomal maintenance or for integration into a host genome.

[065] The term "transgene" as used herein is a nucleic acid sequence that is incorporated into a transgenic organism. A "transgene" can contain one or more transcriptional regulatory sequences, and other sequences, such as introns, that may be useful for expressing or secreting the nucleic acid or fusion protein it encodes.

[066] The term "cDNA" as used herein is intended to include all nucleic acids that share the sequence elements of mature mRNA species, where sequence elements are exons and 3' and 5' non-coding regions. Generally, mRNA species have contiguous exons, the intervening introns having been removed by nuclear RNA splicing to create a continuous open reading frame encoding a protein.

[067] The term "splice variant" refers to all types of RNAs transcribed from a given gene that when processed collectively encode plural protein isoforms. The term "alternative splicing" and related terms refer to all types of RNA processing that lead to expression of plural protein isoforms from a single gene. Some genes are first transcribed as long mRNA precursors that are then shortened by a series of processing steps to produce the mature mRNA molecule. One of these steps is RNA splicing, in which the intron sequences are removed from the mRNA precursor. A cell can splice the primary transcript in different ways, making different "splice variants," and thereby making different polypeptide chains from the same gene, or from the same mRNA molecule. Splice variants can include, for example, exon insertions, exon extensions, exon truncations, exon deletions, alternatives in the 5' untranslated region and alternatives in the 3' untranslated region.

[068] "Oligonucleotide" may generally refer to polynucleotides of between about 5 and about 100 nucleotides of single-or double-stranded nucleic acids. For the purposes of this disclosure, there is no upper limit to the length of an oligonucleotide. Oligonucleotides are also known as oligomers or oligos and can be isolated from genes, or chemically synthesized by methods known in the art.

[069] "Nucleic acid composition" as used herein is a composition comprising a nucleic acid sequence, including one having an open reading frame that encodes a polypeptide and is capable, under appropriate conditions, of being expressed as a polypeptide. The term includes, for example, vectors, including plasmids, cosmids, viral vectors (*e.g.*, retrovirus vectors such as lentivirus, adenovirus, and the like), human, yeast, bacterial, P1-derived artificial chromosomes (HAC's, YAC's, BAC's, PAC's, etc), and mini-chromosomes, *in vitro* host cells, *in vivo* host cells, tissues, organs, allogenic or congenic grafts or transplants, multicellular organisms, and chimeric, genetically modified, or transgenic animals comprising a subject nucleic acid sequence.

[070] An "isolated," "purified," or "substantially isolated" polynucleotide, or a polynucleotide in "substantially pure form," in "substantially purified form," in "substantial purity," or as an "isolate," is one that is substantially free of the sequences with which it is

associated in nature, or other nucleic acid sequences that do not include a sequence or fragment of the subject polynucleotides. By substantially free is meant that less than about 90%, less than about 80%, less than about 70%, less than about 60%, or less than about 50% of the composition is made up of materials other than the isolated polynucleotide. For example, the isolated polynucleotide is at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% free of the materials with which it is associated in nature. For example, an isolated polynucleotide may be present in a composition wherein at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 97%, at least about 99% of the total macromolecules (for example, polypeptides, fragments thereof, polynucleotides, fragments thereof, lipids, polysaccharides, and oligosaccharides) in the composition is the isolated polynucleotide. Where at least about 99% of the total macromolecules is the isolated polynucleotide, the polynucleotide is at least about 99% pure, and the composition comprises less than about 1% contaminant. As used herein, an "isolated," "purified" or "substantially isolated" polynucleotide, or a polynucleotide in "substantially pure form," in "substantially purified form," in "substantial purity," or as an "isolate," also refers to recombinant polynucleotides, modified, degenerate and homologous polynucleotides, and chemically synthesized polynucleotides, which, by virtue of origin or manipulation, are not associated with all or a portion of a polynucleotide with which it is associated in nature, are linked to a polynucleotide other than that to which it is linked in nature, or do not occur in nature. For example, the subject polynucleotides are generally provided as other than on an intact chromosome, and recombinant embodiments are typically flanked by one or more nucleotides not normally associated with the subject polynucleotide on a naturally-occurring chromosome.

[071] The terms "polypeptide," "peptide," and "protein," used interchangeably herein, refer to a polymeric form of amino acids of any length, which can include naturally-occurring amino acids, coded and non-coded amino acids, chemically or biochemically modified, derivatized, or designer amino acids, amino acid analogs, peptidomimetics, and depsipeptides, and polypeptides having modified, cyclic, bicyclic, depsicyclic, or depsibicyclic peptide backbones. The term includes single chain protein as well as multimers. The term also includes conjugated proteins, fusion proteins, including, but not limited to, GST fusion proteins, fusion proteins with a heterologous amino acid sequence, fusion proteins with heterologous and homologous leader sequences, fusion proteins with or

without N-terminal methionine residues, pegylated proteins, and immunologically tagged proteins. Also included in this term are variations of naturally occurring proteins, where such variations are homologous or substantially similar to the naturally occurring protein, as well as corresponding homologs from different species. Variants of polypeptide sequences include insertions, additions, deletions, or substitutions compared with the subject polypeptides. The term also includes peptide aptamers.

[072] The novel polypeptides herein include amino acid sequences encoded by an open reading frame (ORF) as shown in SEQ. ID. NOS. 188-374, described in greater detail below, including the full length protein and fragments thereof, particularly biologically active fragments and/or fragments corresponding to functional domains, *e.g.*, a signal peptide or leader sequence, an enzyme active site, including a cleavage site and an enzyme catalytic site, a domain for interaction with other protein(s), a domain for binding DNA, a regulatory domain, a consensus domain that is shared with other members of the same protein family, such as a kinase family or an immunoglobulin family; an extracellular domain that may act as a target for antibody production or that may be cleaved to become a soluble receptor or a ligand for a receptor; an intracellular fragment of a transmembrane protein that participates in signal transduction; a transmembrane domain of a transmembrane protein that may facilitate water or ion transport; a sequence associated with cell survival and/or cell proliferation; a sequence associated with cell cycle arrest, DNA repair and/or apoptosis; a sequence associated with a disease or disease prognosis, including types of cancer, degenerative disease, inflammatory disease, immunological disease, genetic disease, metabolic disease, and/or bacterial or viral infection; and including fusions of the subject polypeptides to other proteins or parts thereof; modifications of the subject polypeptide, *e.g.*, comprising modified, derivatized, or designer amino acids, modified peptide backbones, and/or immunological tags; as well as intra- and inter-species homologs of the subject polypeptides.

[073] As noted above, a "biologically active" entity, or an entity having "biological activity," is one having structural, regulatory, or biochemical functions of a naturally occurring molecule or any function related to or associated with a metabolic or physiological process. Biologically active polypeptide fragments are those exhibiting activity similar, but not necessarily identical, to an activity of a polypeptide of the present invention. The biological activity can include an improved desired activity, or a decreased undesirable activity. For example, an entity demonstrates biological activity when it participates in a molecular interaction with another molecule, or when it has therapeutic value in alleviating a

disease condition, or when it has prophylactic value in inducing an immune response to the molecule, or when it has diagnostic value in determining the presence of the molecule. A biologically active polypeptide or fragment thereof includes one that can participate in a biological reaction, for example, as a transcription factor that combines with other transcription factors for initiation of transcription, or that can serve as an epitope or immunogen to stimulate an immune response, such as production of antibodies, or that can transport molecules into or out of cells, or that can perform a catalytic activity, for example polymerization or nuclease activity, or that can participate in signal transduction by binding to receptors, proteins, or nucleic acids, activating enzymes or substrates.

[074] A "signal peptide," or a "leader sequence," comprises a sequence of amino acid residues, typically, at the N terminus of a polypeptide, which directs the intracellular trafficking of the polypeptide. Polypeptides that contain a signal peptide or leader sequence typically also contain a signal peptide or leader sequence cleavage site. Such polypeptides, after cleavage at the cleavage sites, generate mature polypeptides, for example, after extracellular secretion or after being directed to the appropriate intracellular compartment.

[075] "Depsipeptides" are compounds containing a sequence of at least two alpha-amino acids and at least one alpha-hydroxy carboxylic acid, which are bound through at least one normal peptide link and ester links, derived from the hydroxy carboxylic acids. "Linear depsipeptides" can comprise rings formed through S-S bridges, or through an hydroxy or a mercapto group of an hydroxy-, or mercapto-amino acid and the carboxyl group of another amino- or hydroxy-acid but do not comprise rings formed only through peptide or ester links derived from hydroxy carboxylic acids. "Cyclic depsipeptides" are peptides containing at least one ring formed only through peptide or ester links, derived from hydroxy carboxylic acids.

[076] An "isolated," "purified," or "substantially isolated" polypeptide, or a polypeptide in "substantially pure form," in "substantially purified form," in "substantial purity," or as an "isolate," is one that is substantially free of the materials with which it is associated in nature or other polypeptide sequences that do not include a sequence or fragment of the subject polypeptides. By substantially free is meant that less than about 90%, less than about 80%, less than about 70%, less than about 60%, or less than about 50% of the composition is made up of materials other than the isolated polypeptide. For example, the isolated polypeptide is at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 97%, or at least about 99%



free of the materials with which it is associated in nature. For example, an isolated polypeptide may be present in a composition wherein at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% of the total macromolecules (for example, polypeptides, fragments thereof, polynucleotides, fragments thereof, lipids, polysaccharides, and oligosaccharides) in the composition is the isolated polypeptide. Where at least about 99% of the total macromolecules is the isolated polypeptide, the polypeptide is at least about 99% pure, and the composition comprises less than about 1% contaminant. As used herein, an "isolated," "purified," or "substantially isolated" polypeptide, or a polypeptide in "substantially pure form," in "substantially purified form," in "substantial purity," or as an "isolate," also refers to recombinant polypeptides, modified, tagged and fusion polypeptides, and chemically synthesized polypeptides, which by virtue of origin or manipulation, are not associated with all or a portion of the materials with which they are associated in nature, are linked to molecules other than that to which they are linked in nature, or do not occur in nature.

[077] Detection methods of the invention can be qualitative or quantitative. Thus, as used herein, the terms "detection," "identification," "determination," and the like, refer to both qualitative and quantitative determinations, and include "measuring." For example, detection methods include methods for detecting the presence and/or level of polynucleotide or polypeptide in a biological sample, and methods for detecting the presence and/or level of biological activity of polynucleotide or polypeptide in a sample.

[078] As used herein, the term "array" or "microarray" may be used interchangeably and refers to a collection of plural biological molecules such as nucleic acids, polypeptides, or antibodies, having locatable addresses that may be separately detectable. Generally, "microarray" encompasses use of sub microgram quantities of biological molecules. The biological molecules may be affixed to a substrate or may be in solution or suspension. The substrate can be porous or solid, planar or non-planar, unitary or distributed, such as a glass slide, a 96 well plate, with or without the use of microbeads or nanobeads. As such, the term "microarray" includes all of the devices referred to as microarrays in Schena, 1999; Bassett et al., 1999; Bowtell, 1999; Brown and Botstein, 1999; Chakravarti, 1999; Cheung et al., 1999; Cole et al., 1999; Collins, 1999; Debouck and Goodfellow, 1999; Duggan et al., 1999; Hacia, 1999; Lander, 1999; Lipshutz et al., 1999; Southern, et al., 1999; Schena, 2000; Brenner et al, 2000; Lander, 2001; Steinhaur et al., 2002; and Espejo et al, 2002. Nucleic acid microarrays

include both oligonucleotide arrays (DNA chips) containing expressed sequence tags ("ESTs") and arrays of larger DNA sequences representing a plurality of genes bound to the substrate, either one of which can be used for hybridization studies. Protein and antibody microarrays include arrays of polypeptides or proteins, including but not limited to, polypeptides or proteins obtained by purification, fusion proteins, and antibodies, and can be used for specific binding studies (Zhu and Snyder, 2003; Houseman et al., 2002; Schaeferling et al., 2002; Weng et al., 2002; Winssinger et al., 2002; Zhu et al., 2001; Zhu et al. 2001; and MacBeath and Schreiber, 2000).

[079] A "nucleic acid hybridization reaction" is one in which single strands of DNA or RNA randomly collide with one another, and bind to each other only when their nucleotide sequences have some degree of complementarity. The solvent and temperature conditions can be varied in the reactions to modulate the extent to which the molecules can bind to one another. Hybridization reactions can be performed under different conditions of "stringency." The "stringency" of a hybridization reaction as used herein refers to the conditions (*e.g.*, solvent and temperature conditions) under which two nucleic acid strands will either pair or fail to pair to form a "hybrid" helix.

[080] " $T_m$ " is the temperature in degrees Celsius at which 50% of a polynucleotide duplex made of complementary strands of nucleic acids that are hydrogen bonded in an anti-parallel direction by Watson-Crick base pairing dissociate into single strands under conditions of the hybridization reaction.  $T_m$  can be predicted according to a standard formula, such as:  $T_m = 81.5 + 16.6 \log[X^+] + 0.41 (\%G/C) - 0.61 (\%F) - 600/L$ , where  $[X^+]$  is the cation concentration (usually sodium ion,  $Na^+$ ) in mol/L; ( $\%G/C$ ) is the number of G and C residues as a percentage of total residues in the duplex; ( $\%F$ ) is the percent formamide in solution (wt/vol); and L is the number of nucleotides in each strand of the paired nucleic acids.

[081] A "buffer" is a system that tends to resist change in pH when a given increment of hydrogen ion or hydroxide ion is added. Buffered solutions contain conjugate acid-base pairs. Any conventional buffer can be used with the inventions herein including but not limited to, for example, Tris, phosphate, imidazole, and bicarbonate.

[082] A "library" of polynucleotides comprises a collection of sequence information of a plurality of polynucleotide sequences, which information is provided in either biochemical form (*e.g.*, as a collection of polynucleotide molecules), or in electronic form

(*e.g.*, as a collection of polynucleotide sequences stored in a computer-readable form, as in a computer-based system, a computer data file, and/or as part of a computer program).

[083] A "library" of polypeptides comprises a collection of sequence information of a plurality of polypeptide sequences, which information is provided in, *e.g.*, a collection of polypeptide sequences stored in a computer-readable form, as in a computer-based system, a computer data file, and/or as part of a computer program.

[084] "Media" refers to a manufacture, other than an isolated nucleic acid molecule, that contains the sequence information of the present invention. Such a manufacture provides the genome sequence or a subset thereof in a form that can be examined by means not directly applicable to the sequence as it exists in a nucleic acid, *e.g.*, with computer-readable media comprising data storage structures. Such media include, but are not limited to: magnetic storage media, such as a floppy disc, a hard disc storage medium, and a magnetic tape; optical storage media such as CD-ROM; electrical storage media such as RAM and ROM; and hybrids of these categories such as magnetic/optical storage media.

[085] "Recorded" refers to a process for storing information on computer readable media, using any such methods as known in the art.

[086] As used herein, "a computer-based system" refers to the hardware means, software means, and data storage means used to analyze the nucleotide sequence information of the present invention. The minimum hardware of the computer-based systems of the present invention comprises a central processing unit (CPU), input means, output means, and data storage means. A skilled artisan can readily appreciate that any one of the currently available computer-based systems are suitable for use in the present invention. The data storage means can comprise any manufacture comprising a recording of the present sequence information as described above, or a memory access means that can access such a manufacture.

[087] "Search means" refers to one or more programs implemented on the computer-based system, to compare a target sequence or target structural motif, or expression levels of a polynucleotide in a sample, with the stored sequence information. A variety of known algorithms are publicly known and commercially available, *e.g.*, MacPattern (EMBL), BLAST, BLASTN and BLASTX (NCBI), gapped BLAST, BLAZE, the Wise package, FASTX, Clustalw, FASTA, FASTA3, Align0, Toffee, BestFit, FastDB, and TeraBLAST (TimeLogic, Crystal Bay, Nevada). Search means can be used to identify fragments or regions of the genome that match a particular target sequence or target motif, for example,

based on sequence similarity, for example, to identify open reading frames (ORFs) within the genome that contain homology to ORFs from other organisms.

[088] "Sequence similarity," "sequence homology," "homology," "sequence identity," and "percent sequence identity," used interchangeably herein, describe the degree of relatedness between two polynucleotide or polypeptide sequences. In general, "identity" means the exact match-up of two or more nucleotide sequences or two or more amino acid sequences, where the nucleotide or amino acids being compared are the same. Also, in general, "similarity" or "homology" means the exact match-up of two or more nucleotide sequences or two or more amino acid sequences, where the nucleotide or amino acids being compared are either the same or possess similar chemical and/or physical properties. The terms also refer to the percentage of the "aligned" bases (for the polynucleotides) or amino acid residues (for the polypeptides) that are identical when the sequences are aligned. Sequences can be aligned in a number of different ways and sequence similarity can be determined in a number of different ways. For example, the bases or amino acid residues of one sequence can be aligned to a gap in the other sequence, or they can be aligned only to another base or amino acid residue in the other sequence. A gap can range anywhere from one nucleotide, base, or amino acid residue to multiple exons in length, up to any number of nucleotides or amino acid residues. Further, sequences can be aligned such that nucleotides (or bases) align with nucleotides, nucleotides align with amino acid residues, or amino acid residues align with amino acid residues.

[089] By "homolog" is meant a protein having at least about 35%, at least about 40%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95%, or higher, amino acid sequence identity to the reference polypeptide, as measured with the "GAP" program (part of the Wisconsin Sequence Analysis Package available through the Genetics Computer Group, Inc. (Madison WI)), where the parameters are: Gap weight:12; length weight:4. In many embodiments of interest, homology will be at least about 75%, at least about 80%, or at least 85%, where in certain embodiments of interest, homology will be as high as about 90%.

[090] A "target sequence" can be any polynucleotide or amino acid sequence of six or more contiguous nucleotides or two or more amino acids, for example, from about 5 or from about 10 to about 100 amino acids, or from about 15 or from about 30 to about 300 nucleotides. A variety of comparing means can be used to accomplish comparison of sequence information from a sample (*e.g.*, to analyze target sequences, target motifs, or

relative expression levels) with the data storage means. A skilled artisan can readily recognize that any one of the publicly available homology search programs can be used as the search means for the computer based systems of the present invention to accomplish comparison of target sequences and motifs. Computer programs to analyze expression levels in a sample and in controls are also known in the art. A "target sequence" includes an "antibody target sequence," which refers to an amino acid sequence that can be used as an immunogen for injection into animals for production of antibodies or for screening against a phage display or antibody library for identification of binding partners.

[091] A "target structural motif," or "target motif," refers to any rationally selected sequence or combination of sequences in which the sequence(s) are chosen based on a three-dimensional configuration that is formed upon the folding of the target motif, or on consensus sequences of regulatory or active sites. There are a variety of target motifs known in the art. Protein target motifs include, but are not limited to, enzyme active sites and signal sequences. Nucleic acid target motifs include, but are not limited to, hairpin structures, promoter sequences, and other expression elements such as binding sites for transcription factors.

[092] A "matrix" is a geometric network of antibody molecules and their antigens, as found in immunoprecipitation and flocculation reactions. An antibody matrix can exist in solution or on a solid phase support.

[093] The term "binds specifically," in the context of antibody binding, refers to high avidity and/or high affinity binding of an antibody to a specific polypeptide, or more accurately, to an epitope of a specific polypeptide. Antibody binding to such epitope on a polypeptide can be stronger than binding of the same antibody to any other epitopes, particularly other epitopes that can be present in molecules in association with, or in the same sample as the polypeptide of interest. For example, when an antibody binds more strongly to one epitope than to another, adjusting the binding conditions can result in antibody binding almost exclusively to the specific epitope and not to any other epitopes on the same polypeptide, and not to any other polypeptide, which does not comprise the epitope. Antibodies that bind specifically to a subject polypeptide may be capable of binding other polypeptides at a weak, yet detectable, level (*e.g.*, 10% or less of the binding shown to the polypeptide of interest). Such weak binding, or background binding, is readily discernible from the specific antibody binding to a subject polypeptide, *e.g.*, by use of appropriate

controls. In general, antibodies of the invention bind to a specific polypeptide with a binding affinity of  $10^{-7}$  M or greater (*e.g.*,  $10^{-8}$  M,  $10^{-9}$  M,  $10^{-10}$ ,  $10^{-11}$ , etc.).

[094] A recombinant vector or construct that includes a nucleic acid of the invention is useful for propagating a nucleic acid in a host cell; such vectors are known as "cloning vectors." Vectors can transfer nucleic acid between host cells derived from disparate organisms; these are known in the art as "shuttle vectors." Vectors can also insert a subject nucleic acid into a host cell's chromosome; these are known in the art as "insertion vectors." Vectors can express either sense or antisense RNA transcripts of the invention *in vitro* (*e.g.*, in a cell-free system or within an *in vitro* cultured host cell) or *in vivo* (*e.g.*, in a multicellular plant or animal); these are known in the art as "expression vectors," which can be part of an expression system. Expression vectors can also produce a subject antibody.

[095] The term "host cell" includes an individual cell, cell line, cell culture, or *in vivo* cell, which can be or has been a recipient of any polynucleotides or polypeptides of the invention, for example, a recombinant vector, an isolated polynucleotide, antibody or fusion protein. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology, physiology, or in total DNA, RNA, or polypeptide complement) to the original parent cell due to natural, accidental, or deliberate mutation and/or change. Host cells can be prokaryotic or eukaryotic, including mammalian, insect, amphibian, reptile, crustacean, avian, fish, plant and fungal cells. A host cell includes cells transformed, transfected, transduced, or infected *in vivo* or *in vitro* with a polynucleotide of the invention, for example, a recombinant vector. A host cell which comprises a recombinant vector of the invention may be called a "recombinant host cell."

[096] "Biological sample," "patient sample," "clinical sample" "sample," or "biological specimen," used interchangeably herein, encompasses a variety of sample types obtained from an individual, including biological fluids such as blood, serum, plasma, urine, cerebrospinal fluid, tears, saliva, lymph, dialysis fluid, lavage fluid, semen, and other liquid samples or tissues of biological origin. It includes tissue samples and tissue cultures or cells derived therefrom and the progeny thereof, including cells in culture, cell supernatants, and cell lysates. It includes organ or tissue culture derived fluids, tissue biopsy samples, tumor biopsy samples, stool samples, and fluids extracted from physiological tissues. Cells dissociated from solid tissues, tissue sections, and cell lysates are included. The definition also includes samples that have been manipulated in any way after their procurement, such as by treatment with reagents, solubilization, or enrichment for certain components, such as

polynucleotides or polypeptides. Also included in the term are derivatives and fractions of biological samples. A biological sample can be used in a diagnostic, monitoring, or screening assay.

[097] The terms "individual," "host," "patient," and "subject," used interchangeably herein, refer to a mammal, including, but not limited to, murines, simians, humans, felines, canines, equines, bovines, porcines, ovines, caprines, mammalian farm animals, mammalian sport animals, and mammalian pets. "Mammals" or "mammalian," are used broadly to describe organisms which are within the class mammalia, including the orders carnivore (*e.g.*, dogs and cats), rodentia (*e.g.*, mice, guinea pigs, and rats), and other mammals, including cattle, goats, sheep, cows, horses, rabbits, and pigs, and primates (*e.g.*, humans, chimpanzees, and monkeys).

[098] The terms "agent," "substance," "modulator," and "compound" are used interchangeably herein. These terms refer to a substance that binds to or modulates a level or activity of a subject polypeptide or a level of mRNA encoding a subject protein or nucleic acid, or that modulates the activity of a cell containing the subject protein or nucleic acid. Where the agent modulates a level of mRNA encoding a subject protein, agents include ribozymes, antisense, and RNAi molecules. Where the agent is a substance that modulates a level of activity of a subject polypeptide, agents include antibodies specific for the subject polypeptide, peptide aptamers, small molecules, agents that bind a ligand-binding site in a subject polypeptide, and the like. Antibody agents include antibodies that specifically bind a subject polypeptide and activate the polypeptide, such as receptor-ligand binding that initiates signal transduction; antibodies that specifically bind a subject polypeptide and inhibit binding of another molecule to the polypeptide, thus preventing activation of a signal transduction pathway; antibodies that bind a subject polypeptide to modulate transcription; antibodies that bind a subject polypeptide to modulate translation; as well as antibodies that bind a subject polypeptide on the surface of a cell to initiate ADCC or to initiate cell killing or cell growth. Small molecule agents include those that bind the polypeptide to modulate activity of the polypeptide or cell containing the polypeptide in a similar fashion. The term "agent" also refers to substances that modulate a condition or disorder associated with a subject polynucleotide or polypeptide. Such agents include subject polynucleotides themselves, subject polypeptides themselves, and the like. Agents may be chosen from amongst candidate agents, as defined below.

[009] The terms "candidate agent," "subject agent," or "test agent," used interchangeably herein, encompass numerous chemical classes, typically synthetic, semi-synthetic, or naturally occurring inorganic or organic molecules, small molecules, or macromolecular complexes. Candidate agents can be small organic compounds having a molecular weight of more than about 50 and less than about 2,500 daltons. Candidate agents can comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and can include at least an amine, carbonyl, hydroxyl or carboxyl group, and can contain at least two of the functional chemical groups. The candidate agents can comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Candidate agents are also found among biomolecules, including oligonucleotides, polynucleotides, and fragments thereof, depsipeptides, polypeptides and fragments thereof, oligosaccharides, polysaccharides and fragments thereof, lipids, fatty acids, steroids, purines, pyrimidines, derivatives thereof, structural analogs, modified nucleic acids, modified, derivatized or designer amino acids, or combinations thereof.

[0100] An "agent which modulates a biological activity of a subject polypeptide," as used herein, describes any substance, synthetic, semi-synthetic, or natural, organic or inorganic, small molecule or macromolecular, pharmaceutical or protein, with the capability of altering a biological activity of a subject polypeptide or of a fragment thereof, as described herein. Generally, a plurality of assay mixtures is run in parallel with different agent concentrations to obtain a differential response to the various concentrations. Typically, one of these concentrations serves as a negative control, *i.e.*, at zero concentration or below the level of detection. The biological activity can be measured using any assay known in the art.

[0101] An agent which modulates a biological activity of a subject polypeptide increases or decreases the activity at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 50%, at least about 100%, or at least about 2-fold, at least about 5-fold, or at least about 10-fold or more when compared to a suitable control.

[0102] The term "agonist" refers to a substance that mimics the function of an active molecule. Agonists include, but are not limited to, drugs, hormones, antibodies, and neurotransmitters, as well as analogues and fragments thereof.

[0103] The term "antagonist" refers to a molecule that competes for the binding sites of an agonist, but does not induce an active response. Antagonists include, but are not



limited to, drugs, hormones, antibodies, and neurotransmitters, as well as analogues and fragments thereof.

[0104] The term "receptor" refers to a polypeptide that binds to a specific extracellular molecule and may initiate a cellular response.

[0105] The term "ligand" refers to any molecule that binds to a specific site on another molecule.

[0106] The term "modulate" encompasses an increase or a decrease, a stimulation, inhibition, or blockage in the measured activity when compared to a suitable control. "Modulation" of expression levels includes increasing the level and decreasing the level of an mRNA or polypeptide encoded by a polynucleotide of the invention when compared to a control lacking the agent being tested. In some embodiments, agents of particular interest are those which inhibit a biological activity of a subject polypeptide, and/or which reduce a level of a subject polypeptide in a cell, and/or which reduce a level of a subject mRNA in a cell and/or which reduce the release of a subject polypeptide from a eukaryotic cell. In other embodiments, agents of interest are those that increase a biological activity of a subject polypeptide, and/or which increase a level of a subject polypeptide in a cell, and/or which increase a level of a subject mRNA in a cell and/or which increase the release of a subject polypeptide from a eukaryotic cell.

[0107] An agent that "modulates the level of expression of a nucleic acid" in a cell is one that brings about an increase or decrease of at least about 1.25-fold, at least about 1.5-fold, at least about 2-fold, at least about 5-fold, at least about 10-fold, or more in the level (*i.e.*, an amount) of mRNA and/or polypeptide following cell contact with a candidate agent compared to a control lacking the agent.

[0108] "Modulating a level of active subject polypeptide" includes increasing or decreasing activity of a subject polypeptide; increasing or decreasing a level of active polypeptide protein; increasing or decreasing a level of mRNA encoding active subject polypeptide, and increasing or decreasing the release of subject polypeptide for a eukaryotic cell. In some embodiments, an agent is a subject polypeptide, where the subject polypeptide itself is administered to an individual. In some embodiments, an agent is an antibody specific for a subject polypeptide. In some embodiments, an agent is a chemical compound such as a small molecule that may be useful as an orally available drug. Such modulation includes the recruitment of other molecules that directly effect the modulation. For example, an antibody that modulates the activity of a subject polypeptide that is a receptor on a cell surface may

bind to the receptor and fix complement, activating the complement cascade and resulting in lysis of the cell.

[0109] The term "over-expressed" refers to a state wherein there exists any measurable increase over normal or baseline levels. For example, a molecule that is over-expressed in a disorder is one that is manifest in a measurably higher level compared to levels in the absence of the disorder.

[0110] A "stem cell" is a pluripotent or multipotent cell with the ability to self-renew, to remain undifferentiated, and to become differentiated. Stem cells can divide without limit, at least for the lifetime of the animal in which they naturally reside. Stem cells are not terminally differentiated, *i.e.*, they are not at the end of a pathway of differentiation. When a stem cell divides, each daughter cell can either remain a stem cell or it can embark on a course that leads to terminal differentiation.

[0111] An "embryonic stem cell" is a stem cell that is present in or isolated from an embryo. An "adult stem cell" is a stem cell that is present in or isolated from an adult. Either can be pluripotent, having the capacity to differentiate into each and every cell present in the organism, or multipotent, with the ability to differentiate into more than one cell type. Embryonic stem cells derived from the inner cell mass of the embryo can act as pluripotent cells when placed into host blastocysts. Adult stem cells are more frequently multipotent than pluripotent; examples of multipotent adult stem cells include hematopoietic stem cells, peripheral nervous system stem cells, central nervous system stem cells, myogenic stem cells, and mesenchymal stem cells.

[0112] A "mesenchymal stem cell" (MSC) is an adult pluripotent stem cell progenitor of multiple mesenchymal lineages, including bone, cartilage, muscle, fat tissue, marrow stroma, and astrocytes. Mesenchyme is embryonic tissue of mesodermal origin, *i.e.*, tissue that derives from the middle of three germ layers. The mesenchyme is populated by mesenchymal cells, which are typically stellate or fusiform in shape. The embryonic mesoderm gives rise to the musculoskeletal, blood, vascular, and urogenital systems, as well as connective tissue, *i.e.*, the dermis.

[0113] A "hematopoietic" cell is a cell involved in the process of hematopoiesis, *i.e.*, the process of forming mature red and white blood cells from precursor cells. In the adult, hematopoiesis takes place in the bone marrow. Earlier in development, hematopoiesis takes place at different sites during different stages of development; primitive blood cells arise in the yolk sac, and later, blood cells are formed in the liver, spleen, and bone marrow.

Hematopoiesis undergoes complex regulation, including regulation by hormones, *e.g.*, erythropoietin; growth factors, *e.g.*, colony stimulating factors; and cytokines, *e.g.*, interleukins. While the B-lymphocytic component of white blood cells matures in the bone marrow, the T-lymphocytic component of white blood cells matures in the thymus.

[0114] "Differentiation" is a progressive developmental change to a more specialized form or function. Cell differentiation is the process a cell undergoes as it matures to become an overtly specialized cell type. Differentiated cells have distinct characteristics, perform specific functions, and are less likely to divide than their less differentiated counterparts. An "undifferentiated" cell, *e.g.*, an immature, embryonic, or primitive cell, typically has a non-specific appearance, may perform multiple, non-specific activities, and may perform poorly, if at all, in functions typically performed by differentiated cells.

[0115] "Dedifferentiation" is a process by which a mature cell returns to a less mature state. A "dedifferentiated cell" is one that has fewer characteristics of differentiation than it possesses at an earlier point in time. A "dedifferentiated state" is one in which a mature cell has returned or is returning to a less differentiated state, *e.g.*, as in some cancers.

[0116] A "differentiation factor" is a factor that induces a cell to undergo a change in the direction of an overtly specialized cell type. An "anti-differentiation factor" is a factor that prevents or inhibits a cell from undergoing a change in the direction toward an overtly specialized cell type.

[0117] A "co-factor" is a molecule that acts in concert with another substance to bring about certain effects.

[0118] A "lymphokine" is a cytokine produced by a leukocyte, which acts upon another cell. Examples include interleukins, interferon-alpha, tumor necrosis factor-alpha, and granulocyte/monocyte colony-stimulating factor.

[0119] An "anti-inflammatory molecule" is a molecule that can diminish, eliminate, or prevent a response to injury or infection. For example, an antihistamine can counteract the effect of the inflammatory mediator histamine.

[0120] An "anti-cancer molecule" is a molecule that can diminish, eliminate, or prevent the effects of cancer. It includes pharmaceuticals and antibodies.

[0121] An "apoptotic molecule" is a molecule that induces a cell to move towards apoptosis, or programmed cell death. Normally functioning cells undergo apoptosis when their age or their state of health so dictates. Apoptosis is an active process requiring metabolic activity by the dying cell, often characterized by cleavage of the DNA into

fragments. Cells that die by apoptosis do not generally elicit the inflammatory response associated with necrosis. Cancer cells do not typically undergo normal apoptosis.

[0122] First and second therapeutic molecules working in "conjunction" means they work in association with one another to achieve a therapeutic effect.

[0123] First and second heterologous nucleic acid sequences that "interact" with one another means they have an effect on one another such that one of the sequences influences the other. Either may act upon the other, or both may act upon each other.

[0124] A "promoter" is a region of DNA that binds RNA polymerase before initiating the transcription of DNA into RNA. The nucleotide at which transcription begins is designated +1; nucleotides are numbered from this reference point. Negative numbers indicate upstream nucleotides and positive numbers indicate downstream nucleotides. The promoter directs the RNA polymerase to bind to DNA, to open the DNA helix, and to begin RNA synthesis. Some promoters are "constitutive," and direct transcription in the absence of regulatory influences. Some promoters are "tissue specific," and initiate transcription exclusively or selectively in one or a few tissue types. Some promoters are "inducible," and effect gene transcription under the influence of an inducer. Induction can occur, *e.g.*, as the result of a physiologic response, a response to outside signals, or as the result of artificial manipulation.

[0125] A "knockout" mouse is a mouse in which a normal functional gene has been replaced by a non-functional form of the gene, and the function of that particular gene is eliminated. They are typically produced by transplanting embryonic stem cells heterozygous for a knockout mutation in a gene of interest and homozygous for a marker gene, *e.g.*, black coat color into the blastocoel cavity of embryos that are homozygous for an alternate marker, *e.g.*, white coat color. The early embryos then are implanted into a pseudopregnant female. Some of the resulting progeny are chimeras, indicated by the phenotype produced by the marker, *e.g.*, a black and white coat. Chimeric mice are backcrossed to mice with the alternate marker. Progeny from this mating that display the marker present in the mice with the gene of interest (*e.g.*, black coat) have embryonic stem-derived cells in their germ line; DNA analysis of these mice can identify the mice heterozygous for the null allele of the gene of interest, *i.e.*, the "knockout" allele. Intercrossing these heterozygous mice produces mice homozygous for the disrupted allele, *i.e.*, "knockout" mice (Capecchi, 1989).

[0126] Gene "knockout" produces model systems for studying inherited human diseases, investigating the nature of genetic diseases and the efficacy of different types of

treatment, and for developing effective gene therapies to cure these diseases. For example, a "knockout" line of mutant mice homozygous for a null allele of the cystic fibrosis transmembrane regulator gene, demonstrates symptoms similar to those of humans with cystic fibrosis. These mice provide a model system for studying this genetic disease and developing effective therapies.

[0127] A "transgenic mouse" is a mouse that has stably incorporated one or more genes from another cell or organism and can pass them on to successive generations. Transgenic mice with an exogenous DNA sequence of interest integrated into its DNA are typically produced by injecting DNA containing a gene of interest into one of the two pronuclei (the male and female haploid nuclei contributed by the parents) of a fertilized mouse egg before they fuse. The injected DNA is randomly integrated into the chromosomes of the diploid zygote. Injected eggs then are transferred to foster mothers in which normal cell growth and differentiation occurs. Some of the progeny will contain the exogenous DNA, and breeding and backcrossing can produce pure transgenic strains homozygous for the transgene (Brinster et al., 1981).

[0128] Transgenic mice are useful for studying various aspects of normal mammalian biology, and also provide a model system for studying disease processes. For example, many forms of cancer are promoted by normal cellular *myc* genes acting in a dominant fashion owing to their misregulated activity. Transgenic mice carrying the *myc* gene develop normally, and form tumors at a high frequency in a subset of cells that express the transgene.

[0129] A "therapeutic factor" encoded by a first heterologous nucleic acid sequence of a modified mesenchymal cell is a factor, excluding a cell survival factor (Mangi et al., 2003; WO 03/073998), that is preventative, palliative, curative, or otherwise useful in treating or ameliorating, or preventing the recurrence of a disease, disorder, syndrome or condition, and is not an anti-cancer agent.

[0130] "Telomerase" is a DNA polymerase enzyme that selectively elongates DNA from the telomere, *i.e.*, the end of a chromosome. Telomeric DNA contains multiple, *e.g.*, hundreds, of tandem repeats of a hexanucleotide sequence. One strand of telomeric DNA is G-rich at the 3' end, and slightly longer than the other strand. Telomeric DNA can form large duplex loops, wherein the single-stranded region at the very end of the structure loops back to form a DNA duplex with another part of the repeated sequence, displacing a part of the original telomeric duplex. This looplike structure is formed and stabilized by specific telomere-binding proteins. These structures protect and mask the end of the chromosome.

[0131] The telomeric looplike structures are generated by telomerase. The telomerase enzyme contains an RNA molecule that serves as the template for elongating the G-rich strand of telomeric DNA. Thus, the enzyme carries the information necessary to generate the telomere sequences. Telomerases also have a protein component, which is related to reverse transcriptases. Telomerases can influence cell aging, and play a role in cellular cancer biology.

[0132] "Tumor necrosis factor" (TNF) encompasses a family of receptor ligands that display pleiotropic effects on normal and malignant cells. Natural induction of TNF is protective, but its overproduction may be detrimental and even lethal to the host. TNF elicits a variety of responses in different cell types. TNF was originally characterized as an antitumor agent and a cytotoxic factor for malignant cells. It subverts the electron transport system of mitochondria to produce oxygen radicals, which can kill malignant cells lacking protective enzymes. TNF also plays a role in the defense against viral, bacterial, and parasitic infections, and in mediating autoimmune responses (Fiers, 1991). TNF inhibitors have been used to treat psoriasis (Weinberg and Saini, 2003).

[0133] "Treatment," "treating," and the like, as used herein, refer to obtaining a desired pharmacologic and/or physiologic effect, covering any treatment of a pathological condition or disorder in a mammal, including a human. The effect may be prophylactic in terms of completely or partially preventing a disorder or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disorder and/or adverse affect attributable to the disorder. That is, "treatment" includes (1) preventing the disorder from occurring or recurring in a subject who may be predisposed to the disorder but has not yet been diagnosed as having it, (2) inhibiting the disorder, such as arresting its development, (3) stopping or terminating the disorder or at least symptoms associated therewith, so that the host no longer suffers from the disorder or its symptoms, such as causing regression of the disorder or its symptoms, for example, by restoring or repairing a lost, missing or defective function, or stimulating an inefficient process, or (4) relieving, alleviating, or ameliorating the disorder, or symptoms associated therewith, where ameliorating is used in a broad sense to refer to at least a reduction in the magnitude of a parameter, such as inflammation, pain, and/or tumor size.

[0134] A "pharmaceutically acceptable carrier," "pharmaceutically acceptable diluent," or "pharmaceutically acceptable excipient," or "pharmaceutically acceptable vehicle," used interchangeably herein, refer to a non-toxic solid, semisolid or liquid filler,

diluent, encapsulating material or formulation auxiliary of any conventional type. A pharmaceutically acceptable carrier is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the carrier for a formulation containing polypeptides would not normally include oxidizing agents and other compounds that are known to be deleterious to polypeptides. Suitable carriers include, but are not limited to, water, dextrose, glycerol, saline, ethanol, and combinations thereof. The carrier can contain additional agents such as wetting or emulsifying agents, pH buffering agents, or adjuvants which enhance the effectiveness of the formulation. Adjuvants of the invention include, but are not limited to Freund's, Montanide ISA Adjuvants [Seppic, Paris, France], Ribi's Adjuvants (Ribi ImmunoChem Research, Inc., Hamilton, MT), Hunter's TiterMax (CytRx Corp., Norcross, GA), Aluminum Salt Adjuvants (Alhydrogel - Superfos of Denmark/Accurate Chemical and Scientific Co., Westbury, NY), Nitrocellulose-Adsorbed Protein, Encapsulated Antigens, and Gerbu Adjuvant (Gerbu Biotechnik GmbH, Gaiberg, Germany/C-C Biotech, Poway, CA). Topical carriers include liquid petroleum, isopropyl palmitate, polyethylene glycol, ethanol (95%), polyoxyethylene monolaurate (5%) in water, or sodium lauryl sulfate (5%) in water. Other materials such as anti-oxidants, humectants, viscosity stabilizers, and similar agents can be added as necessary. Percutaneous penetration enhancers such as Azone can also be included.

[0135] "Pharmaceutically acceptable salts" include the acid addition salts (formed with the free amino groups of the polypeptide) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, mandelic, oxalic, and tartaric. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, and histidine.

[0136] Compositions for oral administration can form solutions, suspensions, tablets, pills, capsules, sustained release formulations, oral rinses, or powders.

[0137] The term "unit dosage form," as used herein, refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of compounds of the present invention calculated in an "effective amount," that is, a dosage sufficient to produce the desired result or effect in association with a pharmaceutically acceptable carrier. The specifications for the novel unit dosage forms of

the present invention depend on the particular compound employed, the host, and the effect to be achieved, as well as the pharmacodynamics associated with each compound in the host.

### **Compositions**

[0138] The present invention provides novel isolated polynucleotides encoding polypeptides and fragments thereof. The present invention also provides novel isolated polypeptides, fragments thereof, and compositions comprising same. The present invention further provides polynucleotide compositions that can be used to identify the polypeptides.

[0139] The present invention provides recombinant vectors and host cells for use in gene expression, primer pairs for use in hybridizations, computer-based embodiments for use in bioinformatics, and transgenic animals and embryonic stem cell lines for use in mutating and regulating gene expression.

### **Nucleic Acids**

#### *Sequences*

[0140] This invention provides genes encoding proteins, the encoded proteins, and fragments and homologs thereof. It provides human polynucleotide sequences and the corresponding mouse polynucleotide sequences.

[0141] The nucleic acids of the subject invention can encode all or a part of the subject proteins. Double or single stranded fragments can be obtained from the DNA sequence by chemically synthesizing oligonucleotides in accordance with conventional methods, for example by restriction enzyme digestion or polymerase chain reaction (PCR) amplification. The use of the polymerase chain reaction has been described (Saiki et al., 1985) and current techniques have been reviewed (Sambrook et al., 1989; McPherson et al. 2000; Dieffenbach and Dveksler, 1995). For the most part, DNA fragments will be of at least about 5 nucleotides, at least about 8 nucleotides, at least about 10 nucleotides, at least about 15 nucleotides, at least about 18 nucleotides, at least about 20 nucleotides, at least about 25 nucleotides, at least about 30 nucleotides, or at least about 50 nucleotides, at least about 75 nucleotides, or at least about 100 nucleotides. Nucleic acid compositions that encode at least six contiguous amino acids (*i.e.*, fragments of 18 nucleotides or more), for example, nucleic acid compositions encoding at least 8 contiguous amino acids (*i.e.*, fragments of 24 nucleotides or more), are useful in directing the expression or the synthesis of peptides that can be used as immunogens (Lerner, 1982; Shinnick et al., 1983; Sutcliffe et al., 1983).

[0142] In some embodiments, a polynucleotide of the invention comprises a nucleotide sequence of at least about 5, at least about 8, at least about 10, at least about 15, at



least about 18, at least about 20, at least about 25, at least about 30, at least about 50, at least about 75, at least about 100, at least about 150, at least about 200, at least about 250, at least about 300, at least about 350, at least about 400, at least about 450, at least about 500, at least about 550, at least about 600, at least about 650, at least about 700, at least about 750, at least about 800, at least about 850, at least about 900, at least about 950, at least about 1000, at least about 1100, at least about 1200, at least about 1300, at least about 1400, at least about 1500, at least about 1600, at least about 1700, at least about 1800, at least about 1900, at least about 2000, at least about 2100, at least about 2200, at least about 2300, at least about 2400, at least about 2500, at least about 3000, at least about 4000, or at least about 5000 contiguous nucleotides of any one of the sequences shown in SEQ. ID. NOS. 1-187 and 375-484, or the coding region thereof, or a complement thereof.

[0143] In other embodiments, a polynucleotide of the invention has at least about 60%, 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 97%, at least about 98%, or at least about 99% nucleotide sequence identity with a nucleotide sequence, or a fragment thereof, of the coding region of any one of the sequences shown in SEQ. ID. NOS. 1-187 and 375-484, or a complement thereof. These sequence variants include naturally-occurring variants (*e.g.*, SNPs, allelic variants, and homologs from other species), degenerate variants, variants associated with disease or pathological states, and variants resulting from random or directed mutagenesis, as well as from chemical or other modification.

[0144] In some embodiments, a polynucleotide of the invention comprises a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence of at least about 5, at least about 8, at least about 10, at least about 15, at least about 18, at least about 20, at least about 25, at least about 30, at least about 50, at least about 75, at least about 100, at least about 150, at least about 200, at least about 250, at least about 300, at least about 350, at least about 400, at least about 450, at least about 500, at least about 550, at least about 600, at least about 650, at least about 700, at least about 750, at least about 800, at least about 850, at least about 900, at least about 950, or at least about 1000 contiguous amino acids of at least one of the sequences shown in SEQ. ID. NOS. 188-374 (*e.g.*, a polypeptide encoded by at least one of the nucleotide sequences shown in SEQ. ID. NOS. 1-187 and 375-484), up to and including an entire amino acid sequence as shown in SEQ. ID. NOS. 188-374 (or as encoded by at least one of the nucleotide sequences shown in SEQ. ID. NOS. 1-187 and 375-484).

[0145] In some embodiment, the present invention includes the present polynucleotide selected from SEQ. ID. NOS. 1-187 and 375-484, which contain 300 bp of 5' terminus of a protein encoding polynucleotide sequence. Such a polynucleotide is useful for the purposes of clustering gene sequences to determine gene family.

[0146] In further embodiments, a polynucleotide of the invention hybridizes under stringent hybridization conditions to a polynucleotide having the coding region of any one of the sequences shown in SEQ. ID. NOS. 1-187 and 375-484, or a complement thereof.

[0147] The polynucleotides of the invention include those that encode variants of the polypeptide sequences encoded by the polynucleotides of the appendices. In some embodiments, these polynucleotides encode variant polypeptides that include insertions, additions, deletions, or substitutions compared with the polypeptides encoded by the nucleotide sequences shown in SEQ. ID. NOS. 1-187 and 375-484, and in Table 1. Conservative amino acid substitutions include serine/threonine, valine/leucine/isoleucine, asparagine/histidine/glutamine, glutamic acid/aspartic acid, etc. (Gonnet et al., 1992).

[0148] The nucleic acids of the invention include degenerate variants that can be translated, according to the standard genetic code, to provide an amino acid sequence identical to that translated from the nucleic acid sequences herein. For example, synonymous codons include GGG, GGA, GGC, and GGU, each encoding Glycine.

[0149] The nucleic acids of the invention include single nucleotide polymorphisms (SNPs), which occur frequently in eukaryotic genomes (Lander, et al. 2001). The nucleotide sequence determined from one individual of a species can differ from other allelic forms present within the population.

[0150] The nucleic acids of the invention include homologs of the polynucleotides. The source of homologous genes can be any species, *e.g.*, primate species, particularly human; rodents, such as rats, hamsters, guinea pigs, and mice; rabbits, canines, felines; cattle, such as bovines; goats, pigs, sheep, equines, crustaceans, birds, chickens, reptiles, amphibians, fish, insects, plants, fungi, yeast, nematodes, etc. Among mammalian species, *e.g.*, human and mouse, homologs have substantial sequence similarity, *e.g.*, at least about 60% sequence identity, at least about 75% sequence identity, or at least about 80% sequence identity among nucleotide sequences. In many embodiments of interest, homology will be at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 98%, where in certain embodiments of interest homology will be as high as about 99%.

[0151] Modifications in the native structure of nucleic acids, including alterations in the backbone, sugars or heterocyclic bases, have been shown to increase intracellular stability and binding affinity. Among useful changes in the backbone chemistry are phosphorothioates; phosphorodithioates, where both of the non-bridging oxygens are substituted with sulfur; phosphoroamidites; alkyl phosphotriesters and boranophosphates. Achiral phosphate derivatives include 3'-O'-5'-S-phosphorothioate, 3'-S-5'-O-phosphorothioate, 3'-CH<sub>2</sub>-5'-O-phosphonate and 3'-NH-5'-O-phosphoroamidate. Peptide nucleic acids replace the entire ribose phosphodiester backbone with a peptide linkage.

[0152] Sugar modifications are also used to enhance stability and affinity. The  $\alpha$ -anomer of deoxyribose can be used, where the base is inverted with respect to the natural  $\beta$ -anomer. The 2'-OH of the ribose sugar can be altered to form 2'-O- methyl or 2'-O-allyl sugars, which provides resistance to degradation without comprising affinity.

[0153] Modification of the heterocyclic bases must maintain proper base pairing. Some useful substitutions include deoxyuridine for deoxythymidine; 5-methyl-2'-deoxycytidine and 5-bromo-2'-deoxycytidine for deoxycytidine. 5- propynyl-2'-deoxyuridine and 5-propynyl-2'-deoxycytidine have been shown to increase affinity and biological activity when substituted for deoxythymidine and deoxycytidine, respectively.

[0154] A genomic sequence of interest comprises the nucleic acid present between the initiation codon and the stop codon, as defined in the listed sequences, including all of the introns that are normally present in a native chromosome. It can further include the 3' and 5' untranslated regions found in the mature mRNA. It can further include specific transcriptional and translational regulatory sequences, such as promoters, enhancers, etc., including about 1 kb, about 2 kb, and possibly more, of flanking genomic DNA at either the 5' or 3' end of the transcribed region. The genomic DNA can be isolated as a fragment of 100 kbp or smaller; and substantially free of flanking chromosomal sequence. The genomic DNA flanking the coding region, either 3' or 5', or internal regulatory sequences as sometimes found in introns, contains sequences required for proper tissue and stage specific expression.

[0155] Nucleic acid molecules of the invention can comprise heterologous nucleic acid molecules, *i.e.*, nucleic acid molecules other than the subject nucleic acid molecules, of any length. For example, the subject nucleic acid molecules can be flanked on the 5' and/or 3' ends by heterologous nucleic acid molecules of from about 1 nucleotide to about 10 nucleotides, from about 10 nucleotides to about 20 nucleotides, from about 20 nucleotides to about 50 nucleotides, from about 50 nucleotides to about 100 nucleotides, from about 100

nucleotides to about 250 nucleotides, from about 250 nucleotides to about 500 nucleotides, or from about 500 nucleotides to about 1000 nucleotides, or more in length.

[0156] The subject polynucleotides include those that encode fusion proteins comprising the subject polypeptides fused to "fusion partners." For example, the present soluble receptor or ligand can be fused to an immunoglobulin fragment, such as an Fc fragment for stability in circulation or to fix complement. Other polypeptide fragments that have equivalent capabilities as the Fc fragments can also be used herein.

[0157] The isolated nucleic acids of the invention can be used as probes to detect and characterize gross alteration in a genomic locus, such as deletions, insertions, translocations, and duplications, *e.g.*, applying fluorescence *in situ* hybridization (FISH) techniques to examine chromosome spreads (Andreeff et al., 1999). The nucleic acids are also useful for detecting smaller genomic alterations, such as deletions, insertions, additions, translocations, and substitutions (*e.g.*, SNPs).

[0158] When used as probes to detect nucleic acid molecules capable of hybridizing with nucleic acids described in the appendices, the nucleic acid molecules can be flanked by heterologous sequences of any length. When used as probes, a subject nucleic acid can include nucleotide analogs that incorporate labels that are directly detectable, such as radiolabels or fluorophores, or nucleotide analogs that incorporate labels that can be visualized in a subsequent reaction, such as biotin or various haptens. Haptens that are commonly conjugated to nucleotides for subsequent labeling include biotin, digoxigenin, and dinitrophenyl.

[0159] Suitable fluorescent labels include fluorochromes *e.g.*, fluorescein and its derivatives, *e.g.*, fluorescein isothiocyanate (FITC6-carboxyfluorescein (6-FAM), 2',7'-dimethoxy-4',5'-dichloro-6-carboxyfluorescein (JOE), ), 6-carboxy-2',4',7',4,7-hexachlorofluorescein (HEX), 5-carboxyfluorescein (5-FAM); coumarin and its derivatives, *e.g.*, 7-amino-4-methylcoumarin, aminocoumarin; bodipy dyes, such as Bodipy FL; cascade blue; Oregon green; rhodamine dyes, *e.g.*, rhodamine, 6-carboxy-X-rhodamine (ROX), Texas red, phycoerythrin, and tetramethylrhodamine; eosins and erythrosins; cyanine dyes, *e.g.*, allophycocyanin, Cy3 and Cy5 or N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA); macrocyclic chelates of lanthanide ions, *e.g.*, quantum dye, etc; and chemiluminescent molecules, *e.g.*, luciferases.

[0160] Fluorescent labels also include a green fluorescent protein (GFP), *i.e.*, a "humanized" version of a GFP, *e.g.*, wherein codons of the naturally-occurring nucleotide

sequence are changed to more closely match human codon bias; a GFP derived from *Aequoria victoria* or a derivative thereof, *e.g.*, a "humanized" derivative such as Enhanced GFP, which are available commercially, *e.g.*, from Clontech, Inc.; other fluorescent mutants of a GFP from *Aequoria victoria*, *e.g.*, as described in U.S. Patent No. 6,066,476; 6,020,192; 5,985,577; 5,976,796; 5,968,750; 5,968,738; 5,958,713; 5,919,445; 5,874,304; a GFP from another species such as *Renilla reniformis*, *Renilla mulleri*, or *Ptilosarcus guernyi*, as previously described (WO 99/49019; Peelle et al., 2001), "humanized" recombinant GFP (hrGFP) (Stratagene®); any of a variety of fluorescent and colored proteins from Anthozoan species, (*e.g.*, Matz et al., 1999).

[0161] Probes can also contain fluorescent analogs, including commercially available fluorescent nucleotide analogs that can readily be incorporated into a subject nucleic acid. These include deoxyribonucleotides and/or ribonucleotide analogs labeled with Cy3, Cy5, Texas Red, Alexa Fluor dyes, rhodamine, cascade blue, or BODIPY, and the like.

[0162] Suitable radioactive labels include, *e.g.*,  $^{32}\text{P}$ ,  $^{35}\text{S}$ , or  $^3\text{H}$ . For example, probes can contain radiolabeled analogs, including those commonly labeled with  $^{32}\text{P}$  or  $^{35}\text{S}$ , such as  $\alpha$ - $^{32}\text{P}$ -dATP, -dTTP, -dCTP, and dGTP;  $\gamma$ - $^{35}\text{S}$ -GTP and  $\alpha$ - $^{35}\text{S}$ -dATP, and the like.

[0163] Nucleic acids of the invention can also be bound to a substrate. Subject nucleic acids can be attached covalently, attached to a surface of the support or applied to a derivatized surface in a chaotropic agent that facilitates denaturation and adherence, *e.g.*, by noncovalent interactions, or some combination thereof. The nucleic acids can be bound to a substrate to which a plurality of other nucleic acids are concurrently bound, hybridization to each of the plurality of the bound nucleic acids being separately detectable.

[0164] The substrate can be porous or solid, planar or non-planar, unitary or distributed; and the bond between the nucleic acid and the substrate can be covalent or non-covalent. The substrate can be in the form of microbeads or nanobeads. Substrates include, but are not limited to, a membrane, such as nitrocellulose, nylon, positively-charged derivatized nylon; a solid substrate such as glass, amorphous silicon, crystalline silicon, plastics (including *e.g.*, polymethylacrylic, polyethylene, polypropylene, polyacrylate, polymethylmethacrylate, polyvinylchloride, polytetrafluoroethylene, polystyrene, polycarbonate, polyacetal, polysulfone, cellulose acetate, or mixtures thereof).

[0165] The subject nucleic acids include antisense RNA, ribozymes, and RNAi. Further, The nucleic acids of the invention can be used for antisense or RNAi inhibition of

transcription or translation using methods known in the art (Phillips, 1999a; Phillips, 1999b; Hartmann et al., 1999; Stein et al., 1998; Agrawal et al., 1998).

### *Expression Vectors*

[0166] The instant invention further provides host cells, *e.g.*, recombinant host cells, that comprise a subject nucleic acid, host cells that comprise a recombinant vector, and host cells that secrete antibodies of the invention. Subject host cells can be cultured *in vitro*, or can be part of a multicellular organism. Host cells are described in more detail below. The instant invention further provides transgenic plants and non-human animals, as described in more detail below.

[0167] In addition to the plurality of uses described in greater detail in following sections, the subject nucleic acids find use in the preparation of all or a portion of the polypeptides of the subject invention, as described above, using an expression system. For expression, an expression vector can be employed. The expression vector will provide a transcriptional and translational initiation region, which may be inducible, conditionally-active, or constitutive, or tissue-specific, where the coding region is operably linked under the transcriptional control of the transcriptional initiation region, and a transcriptional and translational termination region. These control regions can be native to a gene encoding the subject peptides, or can be derived from heterologous or exogenous sources.

[0168] The subject nucleic acids can also be provided as part of a vector (*e.g.*, a polynucleotide construct comprising an expression cassette), a wide variety of which are known in the art. Vectors include, but are not limited to, plasmids; cosmids; viral vectors; human, yeast, bacterial, P1-derived artificial chromosomes (HAC's, YAC's, BAC's, PAC's, etc.), mini-chromosomes, and the like. Vectors are amply described in numerous publications well known to those in the art (Ausubel, et al.; Jones et al., 1998a; Jones et al., 1998b). Vectors can provide for nucleic acid expression, for nucleic acid propagation, or both.

[0169] Vectors typically include at least one origin of replication, at least one site for insertion of heterologous nucleic acid (*e.g.*, in the form of a polylinker with multiple, tightly clustered, single cutting restriction endonuclease recognition sites), and at least one selectable marker, although some integrative vectors will lack an origin that is functional in the host to be chromosomally modified, and some vectors will lack selectable markers. Vectors are transiently or stably maintained in the cells, usually for a period of at least about one day, at least about several days to at least about several weeks.

[0170] Promoters of the invention can be naturally contiguous or not naturally contiguous to the expressed nucleic acid molecule. The promoters can be inducible, conditionally active (such as the cre-lox promoter), constitutive, and/or tissue specific.

[0171] Prior to vector insertion, the DNA of interest will be obtained substantially free of other nucleic acid sequences. The DNA can be "recombinant," and flanked by one or more nucleotides with which it is not normally associated on a naturally occurring chromosome.

[0172] Expression vectors generally have convenient restriction sites located near the promoter sequence to provide for the insertion of nucleic acid sequences encoding heterologous protein or RNA molecules. A selectable marker operative in the expression system or host can be present. Expression vectors can be used for the production of fusion proteins, where the fusion peptide provides additional functionality, *i.e.*, increased protein synthesis, a leader sequence for secretion, stability, reactivity with defined antisera, or an enzyme marker, *e.g.*,  $\beta$ -galactosidase.

[0173] Expression vectors can be prepared comprising a transcription cassette comprising a transcription initiation region, the gene or fragment thereof, and a transcriptional termination region. Of particular interest is the use of DNA sequences that allow for the expression of functional epitopes or domains, at least about 5, at least about 8, at least about 10, at least about 15, at least about 18, at least about 20, at least about 25, at least about 30, at least about 50, at least about 75, at least about 100, at least about 150, at least about 200, at least about 250, at least about 300, at least about 350, at least about 400, at least about 450, at least about 500, at least about 550, at least about 600, at least about 650, at least about 700, at least about 750, at least about 800, at least about 850, at least about 900, at least about 950, or at least about 1000 amino acids in length, or any of the above-described fragments, up to and including the complete open reading frame of the gene. After introduction of these DNA sequences, the cells containing the vector construct can be selected by means of a selectable marker, and the selected cells expanded and used as expression-competent host cells.

[0174] Host cells can comprise prokaryotes or eukaryotes that express proteins and polypeptides in accordance with conventional methods, the method depending on the purpose for expression. For large scale production of the protein, a unicellular organism, such as *E. coli*, *B. subtilis*, *S. cerevisiae*, insect cells in combination with baculovirus vectors, or cells of a higher organism such as vertebrates, particularly mammals, *e.g.*, COS 7 cells, can be used as

the expression host cells. In some situations, it is desirable to express eukaryotic genes in eukaryotic cells, where the encoded protein will benefit from native folding and post-translational modifications.

[0175] Specific expression systems of interest include plants, bacteria, yeast, insect cells, and mammalian cell-derived expression systems. Representative systems from each of these categories are provided below.

[0176] Expression systems in plants include those described in U.S. Patent No. 6,096,546 and U.S. Patent No. 6,127,145.

[0177] Expression systems in bacteria include those described by Chang et al., 1978; Goeddel et al., 1979; Goeddel et al., 1980; EP 0 036,776; U.S. Patent No. 4,551,433; DeBoer et al., 1983); and Siebenlist et al., 1980.

[0178] Expression systems in yeast include those described by Hinnen et al., 1978; Ito et al., 1983; Kurtz et al., 1986; Kunze et al., 1985; Gleeson et al., 1986; Roggenkamp et al., 1986; Das et al., 1984; De Louvencourt et al., 1983; Van den Berg et al., 1990; Kunze et al., 1985; Cregg et al., 1985; U.S. Patent Nos. 4,837,148 and 4,929,555; Beach and Nurse, 1981; Davidow et al., 1985; Gaillardin et al., 1985; Ballance et al., 1983; Tilburn et al., 1983; Yelton et al., 1984; Kelly and Hynes, 1985; EP 0 244,234; WO 91/00357; and U.S. Patent No. 6,080,559.

[0179] Expression systems for heterologous genes in insects include those described in U.S. Patent No. 4,745,051; Friesen et al., 1986; EP 0 127,839; EP 0 155,476; Vlak et al., 1988; Miller et al., 1988; Carbonell et al., 1988; Maeda et al., 1985; Lebacqz-Verheyden et al., 1988; Smith et al., 1985); Miyajima et al., 1987; and Martin et al., 1988. Numerous baculoviral strains and variants and corresponding permissive insect host cells are described in Luckow et al., 1988, Miller et al., 1986, and Maeda et al., 1985. The insect cell expression system is useful not only for production of heterologous proteins intracellularly, but can be used for expression of transmembrane proteins on the insect cell surfaces. Such insect cells can be used as immunogen for production of antibodies, for example, by injection of the insect cells into mice or rabbits or other suitable animals, for production of antibodies.

[0180] Mammalian expression systems include those described in Dijkema et al., 1985; Gorman et al., 1982; Boshart et al., 1985; and U.S. Patent No. 4,399,216. Additional features of mammalian expression are facilitated as described in Ham and Wallace, 1979; Barnes and Sato, 1980 U.S. Patent Nos. 4,767,704, 4,657,866, 4,927,762, 4,560,655, WO



90/103430, WO 87/00195, and U.S. RE 30,985. Mammalian cell expression systems can also be used for production of antibodies.

[0181] The present polynucleotides can also be used in cell-free expression systems such as bacterial system, *e.g.*, *E. coli* lysate, rabbit reticulocyte lysate system, wheat germ extract system, frog oocyte lysate system, and the like which is conventional in the art. See, for example, WO 00/68412, WO 01/27260, WO 02/24939, WO 02/38790, WO 91/02076, and WO 91/02075.

[0182] When any of the above-referenced host cells, or other appropriate host cells or organisms, are used to replicate and/or express the polynucleotides of the invention, the resulting replicated nucleic acid, RNA, expressed protein or polypeptide, is within the scope of the invention as a product of the host cell or organism.

[0183] Once the gene corresponding to a selected polynucleotide is identified, its expression can be regulated in the gene's native cell types. For example, an endogenous gene of a cell can be regulated by an exogenous regulatory sequence inserted into the genome of the cell at a location that will enhance or reduce expression of the gene corresponding to the subject polypeptide. The regulatory sequence can be designed to integrate into the genome via homologous recombination, as disclosed in U.S. Patent Nos. 5,641,670 and 5,733,761, the disclosures of which are herein incorporated by reference. Alternatively, it can be designed to integrate into the genome via non-homologous recombination, as described in WO 99/15650, the disclosure of which is also herein incorporated by reference. Also encompassed in the subject invention is the production of proteins without manipulating the encoding nucleic acid itself, but rather by integrating a regulatory sequence into the genome of a cell that already includes a gene that encodes the protein of interest; this production method is described in the above-incorporated patent documents.

#### *Isolated Primer Pairs*

[0184] In some embodiments, the invention provides isolated nucleic acids that, when used as primers in a polymerase chain reaction, amplify a subject polynucleotide, or a polynucleotide containing a subject polynucleotide. The amplified polynucleotide is from about 20 to about 50, from about 50 to about 75, from about 75 to about 100, from about 100 to about 125, from about 125 to about 150, from about 150 to about 175, from about 175 to about 200, from about 200 to about 250, from about 250 to about 300, from about 300 to about 350, from about 350 to about 400, from about 400 to about 500, from about 500 to about 600, from about 600 to about 700, from about 700 to about 800, from about 800 to

about 900, from about 900 to about 1000, from about 1000 to about 2000, from about 2000 to about 3000, from about 3000 to about 4000, from about 4000 to about 5000, or from about 5000 to about 6000 nucleotides or more in length.

[0185] The isolated nucleic acids themselves are from about 10 to about 20, from about 20 to about 30, from about 30 to about 40, from about 40 to about 50, from about 50 to about 100, or from about 100 to about 200 nucleotides in length. Generally, the nucleic acids are used in pairs in a polymerase chain reaction, where they are referred to as "forward" and "reverse" primers.

[0186] Thus, in some embodiments, the invention provides a pair of isolated nucleic acid molecules, each from about 10 to about 200 nucleotides in length, the first nucleic acid molecule of the pair comprising a sequence of at least 10 contiguous nucleotides having 100% sequence identity to a nucleic acid sequence as shown in SEQ. ID. NOS. 1-187 and 375-484 and the second nucleic acid molecule of the pair comprising a sequence of at least 10 contiguous nucleotides having 100% sequence identity to the reverse complement of the nucleic acid sequence shown in SEQ. ID. NOS. 1-187 and 375-484, wherein the sequence of the second nucleic acid molecule is located 3' of the nucleic acid sequence of the first nucleic acid molecule shown in SEQ. ID. NOS. 1-187 and 375-484. The primer nucleic acids are prepared using any known method, *e.g.*, automated synthesis, and can be chosen to specifically amplify a cDNA copy of an mRNA encoding a subject polypeptide.

[0187] In some embodiments, the first and/or the second nucleic acid molecules comprise a detectable label. The label can be a radioactive molecule, fluorescent molecule or another molecule, *e.g.*, hapten, as described in detail above. Further, the label can be a two stage system, where the amplified DNA is conjugated to another molecule, *i.e.*, biotin, digoxin, or a hapten, that has a high affinity binding partner, *i.e.*, avidin, antidigoxin, or a specific antibody, respectively, and the binding partner conjugated to a detectable label. The label can be conjugated to one or both of the primers. Alternatively, the pool of nucleotides used in the amplification is labeled, so as to incorporate the label into the amplification product.

[0188] Conditions that increase stringency of both DNA/DNA and DNA/RNA hybridization reactions are widely known and published in the art. See, for example, Sambrook, 1989, and examples provided above. Examples of relevant conditions include (in order of increasing stringency): incubation temperatures of 25°C, 37°C, 50°C, and 68°C; buffer concentrations of 10 x SSC, 6 x SSC, 1 x SSC, 0.1 x SSC (where 1 x SSC is 0.15 M

NaCl and 15 mM citrate buffer); and their equivalents using other buffer systems; formamide concentrations of 0%, 25%, 50%, and 75%; incubation times from 5 minutes to 24 hours; 1, 2, or more washing steps; wash incubation times of 1, 2, or 15 minutes; and wash solutions of 6 x SSC, 1 x SSC, 0.1 x SSC, or deionized water.

[0189] For example, "high stringency conditions" include hybridization in 50% formamide, 5X SSC, 0.2 µg/µl poly(dA), 0.2 µg/µl human cot1 DNA, and 0.5% SDS, in a humid oven at 42°C overnight, followed by successive washes in 1X SSC, 0.2% SDS at 55°C for 5 minutes, followed by washing at 0.1X SSC, 0.2% SDS at 55°C for 20 minutes. Further examples of high stringency conditions include hybridization at 50°C and 0.1×SSC (15 mM sodium chloride/1.5 mM sodium citrate); overnight incubation at 42°C in a solution containing 50% formamide, 1 × SSC (150 mM NaCl, 15 mM sodium citrate), 50 mM sodium phosphate (pH 7.6), 5 × Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1 × SSC at about 65°. High stringency conditions also include aqueous hybridization (*e.g.*, free of formamide) in 6X SSC (where 20X SSC contains 3.0 M NaCl and 0.3 M sodium citrate), 1% sodium dodecyl sulfate (SDS) at 65°C for about 8 hours (or more), followed by one or more washes in 0.2 X SSC, 0.1% SDS at 65°C. Highly stringent hybridization conditions are hybridization conditions that are at least as stringent as any one of the above representative conditions. Other stringent hybridization conditions are known in the art and can also be employed to identify nucleic acids of this particular embodiment of the invention.

[0190] Conditions of "reduced stringency," suitable for hybridization to molecules encoding structurally and functionally related proteins, or otherwise serving related or associated functions, are the same as those for high stringency conditions but with a reduction in temperature for hybridization and washing to lower temperatures (*e.g.*, room temperature or about 22°C to 25°C). For example, moderate stringency conditions include aqueous hybridization (*e.g.*, free of formamide) in 6X SSC, 1% SDS at 65°C for about 8 hours (or more), followed by one or more washes in 2X SSC, 0.1% SDS at room temperature. Low stringency conditions include, for example, aqueous hybridization at 50°C and 6×SSC (0.9 M sodium chloride/0.09 M sodium citrate) and washing at 25°C in 1×SSC (0.15 M sodium chloride/0.015 M sodium citrate).

[0191] The specificity of a hybridization reaction allows any single-stranded sequence of nucleotides to be labeled with a radioisotope or chemical and used as a probe to find a complementary strand, even in a cell or cell extract that contains millions of different

DNA and RNA sequences. Probes of this type are widely used to detect the nucleic acids corresponding to specific genes, both to facilitate the purification and characterization of the genes after cell lysis and to localize them in cells, tissues, and organisms.

[0192] Moreover, by carrying out hybridization reactions under conditions of "reduced stringency," a probe prepared from one gene can be used to find homologous evolutionary relatives - both in the same organism, where the relatives form part of a gene family, and in other organisms, where the evolutionary history of the nucleotide sequence can be traced. A person skilled in the art would recognize how to modify the conditions to achieve the requisite degree of stringency for a particular hybridization.

### *Libraries*

[0193] The polynucleotide libraries of the invention generally comprise a collection of sequence information of a plurality of polynucleotide sequences, where at least one of the polynucleotides has a sequence shown in SEQ. ID. NOS. 1-187 and 375-484. By plurality is meant at least 2, at least 3, or at least all of the sequences in the appendices. The information may be provided in either biochemical form (*e.g.*, as a collection of polynucleotide molecules), or in electronic form (*e.g.*, as a collection of polynucleotide sequences stored in a computer-readable form, as in a computer-based system, a computer data file, and/or as a part of a computer program). The length and number of polynucleotides in the library will vary with the nature of the library, *e.g.*, if the library is an oligonucleotide array, a cDNA array, or a computer database of the sequence information.

[0194] The sequence information contained in either a biochemical or an electronic library of polynucleotides can be used in a variety of ways, *e.g.*, as a resource for gene discovery, as a representation of sequences expressed in a selected cell type (*e.g.*, cell type markers), or as markers of a given disorder or disease state. In general, a disease marker is a representation of a gene product that is present in all cells affected by disease either at an increased or decreased level relative to a normal cell (*e.g.*, a cell of the same or similar type that is not substantially affected by disease). For example, a polynucleotide sequence in a library can be a polynucleotide that represents an mRNA, polypeptide, or other gene product encoded by the polynucleotide, that is either over-expressed or under-expressed in one cell compared to another (*e.g.*, a first cell type compared to a second cell type; a normal cell compared to a diseased cell; a cell not exposed to a signal or stimulus compared to a cell exposed to that signal or stimulus; and the like).

[0195] The nucleotide sequence information of the library can be embodied in any suitable form, *e.g.*, electronic or biochemical forms. For example, a library of sequence information embodied in electronic form comprises an accessible computer data file that may contain the representative nucleotide sequences of genes that are differentially expressed (*e.g.*, over-expressed or under-expressed) as between, *e.g.*, a first cell type compared to a second cell type (*e.g.*, expression in a brain cell compared to expression in a kidney cell); a normal cell compared to a diseased cell (*e.g.*, a non-cancerous cell compared to a cancerous cell); a cell not exposed to an internal or external signal or stimulus compared to a cell exposed to that signal or stimulus (*e.g.*, a cell contacted with a ligand compared to a control cell not contacted with the ligand); and the like. Other combinations and comparisons of cells will be readily apparent to the ordinarily skilled artisan. Biochemical embodiments of the library include a collection of nucleic acid molecules that have the sequences of the genes in the library, where the nucleic acids can correspond to the entire gene in the library or to a fragment thereof, as described in greater detail below.

[0196] Where the library is an electronic library, the nucleic acid sequence information can be present in a variety of media. For example, the nucleic acid sequences of any of the polynucleotides shown in SEQ. ID. NOS. 1-187 and 375-484 can be recorded on computer readable media of a computer-based system, *e.g.*, any medium that can be read and accessed directly by a computer. One of skill in the art can readily appreciate how any of the presently known computer readable mediums can be used to create a manufacture comprising a recording of the present sequence information. Any convenient data storage structure can be chosen, based on the means used to access the stored information. A variety of data processor programs and formats can be used for storage, *e.g.*, word processing text file, database format, etc. In addition to the sequence information, electronic versions of the libraries of the invention can be provided in conjunction or connection with other computer-readable information and/or other types of computer-based files (*e.g.*, searchable files, executable files, etc, including, but not limited to, for example, search program software, etc.).

[0197] By providing the nucleotide sequence in computer readable form in a computer-based system, the information can be accessed for a variety of purposes. Computer software to access sequence information is publicly available. Conventional bioinformatics tools can be utilized to analyze sequences to determine sequence identity, sequence similarity, and gap information. For example, the gapped BLAST (Altschul et al., 1990,

Altschul et al., 1997), and BLAZE (Brutlag et al., 1993) search algorithms on a Sybase system, or the TeraBLAST (TimeLogic, Crystal Bay, Nevada) program optionally running on a specialized computer platform available from TimeLogic, can be used to identify open reading frames (ORFs) within the genome that contain homology to ORFs from other organisms. Homology between sequences of interest can be determined using the local homology algorithm of Smith and Waterman, 1981, as well as the BestFit program (Rechid et al., 1989), and the FastDB algorithm (FastDB, 1988; described in Current Methods in Sequence Comparison and Analysis, Macromolecule Sequencing and Synthesis, Selected Methods and Applications, pp. 127-149, 1988, Alan R. Liss, Inc).

[0198] Alignment programs that permit gaps in the sequence include Clustalw (Thompson et al., 1994), FASTA3 (Pearson, 2000) Align0 (Myers and Miller, 1988), and Toffee (Notredame et al., 2000). Other methods for comparing and aligning nucleotide and protein sequences include, for example, BLASTX (NCBI), the Wise package (Birney and Durbin, 2000), and FASTX (Pearson, 2000). These algorithms determine sequence homology between nucleotide and protein sequences without translating the nucleotide sequences into protein sequences. Other techniques for alignment are also known in the art (Doolittle, et al., 1996; BLAST, available from the National Center for Biotechnology Information; FASTA, available in the Genetics Computing Group (GCG) package, from Madison, Wisconsin, USA, a wholly owned subsidiary of Oxford Molecular Group, Inc.; Schlessinger, 1988a; Schlessinger, 1988b; and Needleman and Wunch, 1970).

[0199] Sequence similarity is calculated based on a reference sequence, which may be a subset of a larger sequence, such as a conserved motif, coding region, flanking region, etc. The reference sequence is usually at least about 18 nt long, at least about 30 nt long, or may extend to the complete sequence that is being compared.

[0200] One parameter for determining percent sequence identity is the percentage of the alignment in the region of strongest alignment between a target and a query sequence. Methods for determining this percentage involve, for example, counting the number of aligned bases of a query sequence in the region of strongest alignment and dividing this number by the total number of bases in the region. For example, 10 matches divided by 11 total residues gives a percent sequence identity of approximately 90.9%. The length of the aligned region is typically at least about 55%, at least about 58%, or at least about 60% of the total sequence length, and can be as great as about 62%, as great as about 64%, and even as great as about 66% of the total sequence length.

[0201] The present invention includes human and mouse polynucleotide and polypeptide sequences that are at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% homologous to the sequences in the appendices, based on using the method of determining sequence identity with the insertion of gaps to detect the maximum degree of sequence identity. In other embodiments of interest, homology will be at least about 80%, at least about 85%, or as high as about 90%.

[0202] A variety of structural formats for the input and output means can be used to input and output the information in the computer-based systems of the present invention. One format for an output means ranks the relative expression levels of different polynucleotides. Such presentation provides a skilled artisan with a ranking of relative expression levels to determine a gene expression profile.

[0203] As discussed above, the library of the invention also encompasses biochemical libraries of the polynucleotides shown in SEQ. ID. NOS. 1-187 and 375-484, *e.g.*, collections of nucleic acids representing the provided polynucleotides. The biochemical libraries can take a variety of forms, *e.g.*, a solution of cDNAs, a pattern of probe nucleic acids stably associated with a surface of a solid support (*i.e.*, an array) and the like. Of particular interest are nucleic acid arrays in which one or more of the polynucleotide sequences shown in SEQ. ID. NOS. 1-187 and 375-484 is represented on the array. A variety of different array formats have been developed and are known to those of skill in the art. The arrays of the subject invention find use in a variety of applications, including gene expression analysis, drug screening, mutation analysis, and the like, as disclosed in the herein-listed exemplary patent documents.

[0204] In addition to the above nucleic acid libraries, analogous libraries of polypeptides are also provided, where the polypeptides of the library will represent at least a portion of the polypeptides encoded by a gene corresponding to one or more of the sequences shown in SEQ. ID. NOS. 1-187 and 375-484.

[0205] Further, analogous libraries of antibodies are also provided, where the libraries comprise antibodies or fragments thereof that specifically bind to at least a portion of at least one of the subject polypeptides. Further, antibody libraries may comprise antibodies or fragments thereof that specifically inhibit binding of a subject polypeptide to its ligand or substrate, or that specifically inhibit binding of a subject polypeptide as a substrate to another molecule. Moreover, corresponding nucleic acid libraries are also provided, comprising polynucleotide sequences that encode the antibodies or antibody fragments described above.

**Polypeptides***Sequences*

[0206] This invention provides novel polypeptides, and related polypeptide compositions. The novel polypeptides of the invention encompass proteins with amino acid sequences as shown in SEQ. ID. NOS. 188-374, or encoded by the nucleic acids having nucleotide sequences shown in SEQ. ID. NOS. 1-187 and 375-484. The subject polypeptides are human polypeptides, fragments thereof, variants (such as splice variants), homologs from other species, and derivatives thereof. In particular embodiments, a polypeptide of the invention has an amino acid sequence substantially identical to the sequence of any polypeptide encoded by a polynucleotide sequence shown in SEQ. ID. NOS. 1-187 and 375-484.

[0207] These polypeptides may reside within the cell, or extracellularly. They may be secreted from the cell, reside in the cytoplasm, in the membranes, or in any of the intracellular organelles, including the nucleus, mitochondria, ribosomes, or storage granules.

[0208] In some embodiments, the present novel polypeptide modulates the cells or tissues of animals, particularly humans, such as, for example, by stimulating, enhancing or inhibiting T or B cell function or the function of other hematopoietic cells or bone marrow cells; modulates adult or embryonic stem cell or precursor cell growth or differentiation; modulates cell function or activity of neuronal cells or other cells of the CNS, heart cells, liver cells, kidney cells, lung cells, pancreatic cells, gastrointestinal cells, spleen cells, breast cells, prostate cells, ovarian cells, and the like.

[0209] In some embodiments, a subject polypeptide is present as a multimer. Multimers include homodimers, homotrimers, homotetramers, and multimers that include more than four monomeric units. Multimers also include heteromultimers, *e.g.*, heterodimers, heterotrimers, heterotetramers, etc. where the subject polypeptide is present in a complex with proteins other than the subject polypeptide. Where the multimer is a heteromultimer, the subject polypeptide can be present in a 1:1 ratio, a 1:2 ratio, a 2:1 ratio, or other ratio, with the other protein(s).

[0210] In addition to the above specifically listed proteins, polypeptides from other species are also provided, including mammals, such as: primates, rodents, *e.g.*, mice, rats, hamsters, guinea pigs; domestic animals, *e.g.*, sheep, pig, horse, cow, goat, rabbit, dog, cat; and humans, as well as non-mammalian species, *e.g.*, avian, reptile and amphibian, insect, crustacean, fish, plant, fungus, and protozoa.



[0211] Also provided are polypeptides that are substantially identical to the at least one amino acid sequence shown in the appendices, or a fragment thereof, whereby substantially identical is meant that the protein has an amino acid sequence identity to the reference sequence of at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 97%, at least about 98%, or at least about 99%.

[0212] The proteins of the subject invention (*e.g.*, polypeptides encoded by the nucleotide sequences shown in SEQ. ID. NOS. 1-187 and 375-484, and polypeptide sequences shown in SEQ. ID. NOS. 188-374) have been separated from their naturally occurring environment and are present in a non-naturally occurring environment. In certain embodiments, the proteins are present in a composition where they are more concentrated than in their naturally occurring environment. For example, purified polypeptides are provided.

[0213] In addition to naturally occurring proteins, polypeptides that vary from naturally occurring forms are also provided. Fusion proteins can comprise a subject polypeptide, or fragment thereof, and a polypeptide other than a subject polypeptide ("the fusion partner") fused in-frame at the N-terminus and/or C-terminus of the subject polypeptide, or internally to the subject polypeptide.

[0214] Suitable fusion partners include, but are not limited to, immunologically detectable proteins (*e.g.*, epitope tags, such as hemagglutinin, FLAG, and c-myc); polypeptides that provide a detectable signal or that serve as detectable markers (*e.g.*, a fluorescent protein, *e.g.*, a green fluorescent protein, a fluorescent protein from an Anthozoan species;  $\beta$ -galactosidase; luciferase; cre recombinase; and the like); polypeptides that provide a catalytic function or induce a cellular response; polypeptides that provide for secretion of the fusion protein from a eukaryotic cell; polypeptides that provide for secretion of the fusion protein from a prokaryotic cell; polypeptides that provide for binding to metal ions (*e.g.*, His<sub>n</sub>, where n = 3-10, *e.g.*, 6His) and structural proteins. Fusion partners can also be those that are able to stabilize the present polypeptide, such as polyethylene glycol ("PEG") and a fragment of an immunoglobulin, such as the Fc fragment of IgG, IgE, IgA, IgM, and/or IgD.

[0215] Detection methods are chosen based on the detectable fusion partner. For example, where the fusion partner provides an immunologically recognizable epitope, an epitope-specific antibody can be used to quantitatively detect the level of polypeptide. In some embodiments, the fusion partner provides a detectable signal, and in these embodiments, the detection method is chosen based on the type of signal generated by the

fusion partner. For example, where the fusion partner is a fluorescent protein, fluorescence is measured.

[0216] Where the fusion partner is an enzyme that yields a detectable product, the product can be detected using an appropriate means. For example,  $\beta$ -galactosidase can, depending on the substrate, yield a colored product that can be detected with a spectrophotometer, and the fluorescent protein luciferase can yield a luminescent product detectable with a luminometer.

[0217] In some embodiments, a polypeptide of the invention comprises at least about 5, at least about 8, at least about 10, at least about 15, at least about 18, at least about 20, at least about 25, at least about 30, at least about 50, at least about 75, at least about 100, at least about 150, at least about 200, at least about 250, at least about 300, at least about 350, at least about 400, at least about 450, at least about 500, at least about 550, at least about 600, at least about 650, at least about 700, at least about 750, at least about 800, at least about 850, at least about 900, at least about 950, or at least about 1000 contiguous amino acid residues of at least one of the sequences according to SEQ. ID. NOS. 188-374, up to and including the entire amino acid sequence.

[0218] Fragments of the subject polypeptides, as well as polypeptides comprising such fragments, are also provided. Fragments of polypeptides of interest will typically be at least about 5, at least about 8, at least about 10, at least about 15, at least about 18, at least about 20, at least about 25, at least about 30, at least about 50, at least about 75, at least about 100, at least about 150, at least about 200, at least about 250, or at least 300 aa in length or longer, where the fragment will have a stretch of amino acids that is identical to the subject protein of at least about 5, at least about 8, at least about 10, at least about 15, at least about 18, at least about 20, at least about 25, at least about 30, or at least about 50 aa in length.

[0219] In some embodiments, fragments exhibit one or more activities associated with a corresponding naturally occurring polypeptide. Fragments find utility in generating antibodies to the full-length polypeptide; and in methods of screening for candidate agents that bind to and/or modulate polypeptide activity. Specific fragments of interest include those with enzymatic activity, those with biological activity including the ability to serve as an epitope or immunogen, and fragments that bind to other proteins or to nucleic acids.

[0220] The invention provides polypeptides comprising such fragments, including, *e.g.*, fusion polypeptides comprising a subject polypeptide fragment fused in frame (directly or indirectly) to another protein (the "fusion partner"), such as the signal peptide of one

protein being fused to the mature polypeptide of another protein. Such fusion proteins are typically made by linking the encoding polynucleotides together in a vector or cassette. Suitable fusion partners include, but are not limited to, immunologically detectable proteins (*e.g.*, epitope tags, such as hemagglutinin, FLAG, and c-myc); polypeptides that provide a detectable signal or that serve as detectable markers (*e.g.*, a fluorescent protein, *e.g.*, a green fluorescent protein, a fluorescent protein from an Anthozoan species;  $\beta$ -galactosidase; luciferase; cre recombinase); polypeptides that provide a catalytic function or induce a cellular response; polypeptides that provide for secretion of the fusion protein from a eukaryotic cell; polypeptides that provide for secretion of the fusion protein from a prokaryotic cell; polypeptides that provide for binding to metal ions (*e.g.*, His<sub>n</sub>, where n = 3-10, *e.g.*, 6His) and structural proteins. Fusion partners can also be those that are able to stabilize the present polypeptide, such as polyethylene glycol ("PEG") and a fragment of an immunoglobulin, such as the Fc fragment of IgG, IgE, IgA, IgM, and/or IgD.

*Polypeptide Preparation.*

[0221] Polypeptides of the invention can be obtained from naturally-occurring sources or produced synthetically. The sources of naturally occurring polypeptides will generally depend on the species from which the protein is to be derived, *i.e.*, the proteins will be derived from biological sources that express the proteins. The subject proteins can also be derived from synthetic means, *e.g.*, by expressing a recombinant gene encoding a protein of interest in a suitable system or host or enhancing endogenous expression, as described in more detail above. Further, small peptides can be synthesized in the laboratory by techniques well known in the art.

[0222] In all cases, the product can be recovered by any appropriate means known in the art. For example, convenient protein purification procedures can be employed (*e.g.*, see Guide to Protein Purification, Deutscher et al., 1990). That is, a lysate can be prepared from the original source, (*e.g.*, a cell expressing endogenous polypeptide, or a cell comprising the expression vector expressing the polypeptide(s)), and purified using HPLC, exclusion chromatography, gel electrophoresis, or affinity chromatography, and the like.

[0223] The invention thus also provides methods of producing polypeptides. Briefly, the methods generally involve introducing a nucleic acid construct into a host cell *in vitro* and culturing the host cell under conditions suitable for expression, then harvesting the polypeptide, either from the culture medium or from the host cell, (*e.g.*, by disrupting the host cell), or both, as described in detail above. The invention also provides methods of producing

a polypeptide using cell-free *in vitro* transcription/translation methods, which are well known in the art, also as provided above.

[0224] Moreover, the invention provides polypeptides, including polypeptide fragments, as targets for therapeutic intervention, including use in screening assays, for identifying agents that modulate polypeptide level and/or activity, and as targets for antibody and small molecule therapeutics, for example, in the treatment of disorders.

#### *Protein Families*

[0225] The sequences of the invention encompass a variety of different types of nucleic acids and polypeptides with different structures and functions. They can encode or comprise polypeptides belonging to different protein families ("Pfam"). The "Pfam" system is an organization of protein sequence classification and analysis, based on conserved protein domains; it can be publicly accessed in a number of ways, for example, at <http://pfam.wustl.edu>. Protein domains are portions of proteins that have a tertiary structure and sometimes have enzymatic or binding activities; multiple domains can be connected by flexible polypeptide regions within a protein. Pfam domains can comprise the N-terminus or the C-terminus of a protein, or can be situated at any point in between. The Pfam system identifies protein families based on these domains and provides an annotated, searchable database that classifies proteins into families (Bateman et al., 2002).

[0226] Sequences of the invention can encode or be comprised of more than one Pfam. Sequences encompassed by the invention include, but are not limited to, the polypeptide and polynucleotide sequences of the molecules shown in the appendices and corresponding molecular sequences found at all developmental stages of an organism. Sequences of the invention can comprise genes or gene segments designated in the appendices, and their gene products, *i.e.*, RNA and polypeptides. They also include variants of those presented in the appendices that are present in the normal physiological state, *e.g.*, variant alleles such as SNPs, splice variants, as well as variants that are affected in pathological states, such as disease-related mutations or sequences with alterations that lead to pathology, and variants with conservative amino acid changes. Some sequences of the invention are categorized below with respect to one or more protein family. Any given sequence can belong to one or more than one category.

[0227] Some sequences of the invention can encode or be comprised of reverse transcriptase (rvt) domains, which are involved in RNA-directed DNA polymerase activity, an enzymatic activity that uses an RNA template to produce DNA for integration into a host

genome (<http://pfam.wustl.edu/cgi-bin/getdesc?name=rvt>). Some sequences of the invention can encode or be comprised of transposase 1 (Transposase\_1) domains, which are characterized by sequences that can excise and/or insert mobile genetic elements such as transposons or insertion sequences, for example, the mariner protein ([http://pfam.wustl.edu/cgi-bin/getdesc?name=Transposase\\_1](http://pfam.wustl.edu/cgi-bin/getdesc?name=Transposase_1)). Some sequences of the invention can encode or be comprised of gag gene protein p24 (core nucleocapsid protein or gag\_p24) domains, which comprise the proteins of the nucleocapsid shell around the RNA of a retrovirus. The gag\_p24, or p24, protein from retroviruses forms the inner protein layer of the nucleocapsid, and performs orchestrated tasks during the assembly, budding, maturation, and infection stages of the viral replication cycle. Gag precursors also function during viral assembly to selectively bind and package strands of genomic RNA. ELISA tests for p24 are among the most commonly used methods to demonstrate virus replication both *in vivo* and *in vitro*. ([http://pfam.wustl.edu/cgi-bin/getdesc?name=Gag\\_p24](http://pfam.wustl.edu/cgi-bin/getdesc?name=Gag_p24)).

[0228] Some sequences of the invention can encode or be comprised of integrase core domains, which mediate the integration of a DNA copy of a viral genome into a host chromosome; *e.g.*, HIV integrase catalyses the incorporation of virally derived DNA into the human genome, presenting a target for the development of new therapeutics for the treatment of AIDS (<http://pfam.wustl.edu/cgi-bin/getdesc?name=rve>). Some sequences of the invention can encode or be comprised of RNase H domains, which are nucleases specific for the RNA strand of an RNA-DNA hybrid that cleaves phosphodiester bonds to produce molecules with 3'-OH and 5'-PO<sub>4</sub> ends (<http://www.sanger.ac.uk/cgi-bin/Pfam/getacc?PF00075>). Antisense deoxyoligonucleotides that trigger RNase H activity can thus be used as cancer therapeutic agents (Crooke, 1996; Curcio et al., 1997).

## Methods

[0229] The present invention provides methods of producing a subject polypeptide and provides antibodies that specifically bind to a subject polypeptide. The present invention further provides screening methods for identifying agents that modulate a level or an activity of a subject polypeptide or polynucleotide. The present invention thus also provides agents that modulate a level or an activity of a subject polypeptide or polynucleotide, as well as compositions, including pharmaceutical compositions, comprising a subject agent.

[0230] The present invention further provides methods for treating disorders such as, for example, cancer and other proliferative disorders, inflammatory and immune disorders, metabolic disorders, and bacterial or viral disorders.

## **Diagnostic and Therapeutic Applications**

### *Screening and Diagnostic Methods*

#### *1. Identifying Biological Molecules that Interact with a Polypeptide*

[0231] Formation of a binding complex between a subject polypeptide and an interacting polypeptide or other macromolecule (*e.g.*, DNA, RNA, lipids, polysaccharides, and the like) can be detected using any known method. Suitable methods include: a yeast two-hybrid system (Zhu et al., 1997; Fields and Song, 1989; U.S. Pat. No. 5,283,173; Chien et al. 1991); a mammalian cell two-hybrid method; a fluorescence resonance energy transfer (FRET) assay; a bioluminescence resonance energy transfer (BRET) assay; a fluorescence quenching assay; a fluorescence anisotropy assay (Jameson and Sawyer, 1995); an immunological assay; and an assay involving binding of a detectably labeled protein to an immobilized protein.

[0232] Immunological assays, and assays involving binding of a detectably labeled protein to an immobilized protein can be performed in a variety of ways. For example, immunoprecipitation assays can be designed such that the complex of protein and an interacting polypeptide is detected by precipitation with an antibody specific for either the protein or the interacting polypeptide.

[0233] FRET detects formation of a binding complex between a subject polypeptide and an interacting polypeptide. It involves the transfer of energy from a donor fluorophore in an excited state to a nearby acceptor fluorophore. For this transfer to take place, the donor and acceptor molecules must be in close proximity (*e.g.*, less than 10 nanometers apart, usually between 10 and 100 Å apart), and the emission spectra of the donor fluorophore must overlap the excitation spectra of the acceptor fluorophore. In these embodiments, a fluorescently labeled subject protein serves as a donor and/or acceptor in combination with a second fluorescent protein or dye.

[0234] Fluorescent proteins can be produced by generating a construct comprising a protein and a fluorescent fusion partner. These are well-known in the art, as described above, including green fluorescent protein (GFP), *i.e.*, a "humanized" version of a GFP, *e.g.*, wherein codons of the naturally-occurring nucleotide sequence are changed to more closely match human codon bias; a GFP derived from *Aequoria victoria* or a derivative thereof, *e.g.*, a "humanized" derivative such as Enhanced GFP, which are available commercially, *e.g.*, from Clontech, Inc.; other fluorescent mutants of a GFP from *Aequoria victoria*, *e.g.*, as described in U.S. Patent No. 6,066,476; 6,020,192; 5,985,577; 5,976,796; 5,968,750; 5,968,738;

5,958,713; 5,919,445; 5,874,304; a GFP from another species such as *Renilla reniformis*, *Renilla mulleri*, or *Ptilosarcus guernyi*, as previously described (WO 99/49019; Peelle et al., 2001), "humanized" recombinant GFP (hrGFP) (Stratagene®); any of a variety of fluorescent and colored proteins from Anthozoan species, (e.g., Matz et al., 1999); as well as proteins labeled with other fluorescent dyes, fluorescein and its derivatives, e.g., fluorescein isothiocyanate (FITC), 6-carboxyfluorescein (6-FAM), 6-carboxy-2',4',7',4',7-hexachlorofluorescein (HEX), 5-carboxyfluorescein (5-FAM), 2',7'-dimethoxy-4',5'-dichloro-6-carboxyfluorescein (JOE); rhodamine dyes, e.g., Texas red, phycoerythrin, tetramethylrhodamine, rhodamine, 6-carboxy-X-rhodamine (ROX); coumarin and its derivatives, e.g., 7-amino-4-methylcoumarin, aminocoumarin; bodipy dyes, such as Bodipy FL; cascade blue; Oregon green; eosins and erythrosins; cyanine dyes, e.g., allophycocyanin, Cy3, Cy5, and N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA); macrocyclic chelates of lanthanide ions, e.g., quantum dye, etc; and chemiluminescent molecules, e.g., luciferases.

[0235] Fluorescent subject proteins can also be generated by producing the subject protein in an auxotrophic strain of bacteria which requires addition of one or more amino acids in the medium for growth. A subject protein-encoding construct that provides for expression in bacterial cells is introduced into the auxotrophic strain, and the bacteria are cultured in the presence of a fluorescent amino acid, which is incorporated into the subject protein produced by the bacterium. The subject protein is then purified from the bacterial culture using standard methods for protein purification.

[0236] BRET is a protein-protein interaction assay based on energy transfer from a bioluminescent donor to a fluorescent acceptor protein. The BRET signal is measured by the ratio of the amount of light emitted by the acceptor to the amount of light emitted by the donor. The ratio of these two values increases as the two proteins are brought into proximity. The BRET assay has been described in the literature (U.S. Patent Nos. 6,020,192; 5,968,750; 5,874,304; Xu, et al. 1999). BRET assays can be performed by analyzing transfer between a bioluminescent donor protein and a fluorescent acceptor protein. Interaction between the donor and acceptor proteins can be monitored by a change in the ratio of light emitted by the bioluminescent and fluorescent proteins. In this application, the subject protein serves as donor and/or acceptor protein.

[0237] Fluorescence anisotropy is a measurement of the rotational mobility of a multi-molecular complex. It can be used to generate information about the binding of one

molecule to another, including the affinity and specificity of binding sites. It can be applied to polypeptides or nucleic acids of the present invention.

[0238] Fluorescence quenching measurements are useful in detecting protein multimerization, such as where the subject protein interacts with at least a second protein and, for example, where multimerization interaction is affected by a test agent. As used herein, the term "multimerization" refers to formation of dimers, trimers, tetramers, and higher multimers of the subject protein. Whether a subject protein forms a complex with one or more additional protein molecules can be determined using any known assay, including assays as described above for interacting proteins. Formation of multimers can also be detected using non-denaturing gel electrophoresis, where multimerized subject protein migrates more slowly than monomeric subject protein. Formation of multimers can also be detected using fluorescence quenching techniques.

[0239] Formation of multimers can also be detected by analytical ultracentrifugation, for example through glycerol or sucrose gradients, and subsequent visualization of a subject protein in gradient fractions by Western blotting or staining of SDS-polyacrylamide gels. Multimers are expected to sediment at defined positions in such gradients. Formation of multimers can also be detected using analytical gel filtration, *e.g.*, in HPLC or FPLC systems, *e.g.*, on columns such as Superdex 200 (Pharmacia Amersham Inc.). Multimers run at defined positions on these columns, and fractions can be analyzed as above. The columns are highly reproducible, allowing one to relate the number and position of peaks directly to the multimerization status of the protein.

## *2. Detecting mRNA Levels and Monitoring Gene Expression*

[0240] The present invention provides methods for detecting the presence of mRNA in a biological sample. The methods can be used, for example, to assess whether a test compound affects gene expression, either directly or indirectly. The present invention provides diagnostic methods to compare the abundance of a nucleic acid with that of a control value, either qualitatively or quantitatively, and to relate the value to a normal or abnormal expression pattern.

[0241] Methods of measuring mRNA levels are known in the art (Pietu, 1996; Zhao, 1995; Soares, 1997; Raval, 1994; Chalifour, 1994; Stolz, 1996; Hong, 1982; McGraw, 1984; WO 97/27317). These methods generally comprise contacting a sample with a polynucleotide of the invention under conditions that allow hybridization and detecting hybridization, if any, as an indication of the presence of the polynucleotide of interest.



Appropriate controls include the use of a sample lacking the polynucleotide mRNA of interest, or the use of a labeled polynucleotide of the same "sense" as a polynucleotide mRNA of interest. Detection can be accomplished by any known method, including, but not limited to, *in situ* hybridization, PCR, RT-PCR, and "Northern" or RNA blotting, or combinations of such techniques, using a suitably labeled subject polynucleotide. A variety of labels and labeling methods for polynucleotides are known in the art and can be used in the assay methods of the invention. A common method employed is use of microarrays which can be purchased or customized, for example, through conventional vendors such as Affymetrix.

[0242] In some embodiments, the methods involve generating a cDNA copy of an mRNA molecule in a biological sample, and amplifying the cDNA using an isolated primer pairs as described above, *i.e.*, a set of two nucleic acid molecules that serve as forward and reverse primers in an amplification reaction (*e.g.*, a polymerase chain reaction). The primer pairs are chosen to specifically amplify a cDNA copy of an mRNA encoding a polypeptide. A detectable label can be included in the amplification reaction, as provided above. Methods using PCR amplification can be performed on the DNA from a single cell, although it is convenient to use at least about  $10^5$  cells.

[0243] The present invention provides methods for monitoring gene expression. Changes in a promoter or enhancer sequence that can affect gene expression can be examined in light of expression levels of the normal allele by various methods known in the art. Methods for determining promoter or enhancer strength include quantifying the expressed natural protein, and inserting the variant control element into a vector with a quantitative reporter gene such as  $\beta$ -galactosidase, luciferase, or chloramphenicol acetyltransferase (CAT).

### 3. *Detecting Polymorphisms and Mutations*

[0244] Biochemical studies can determine whether a sequence polymorphism in a coding region or control region is associated with disease. Disease-associated polymorphisms can include deletion or truncation of the gene, mutations that alter expression level, or mutations that affect protein function, etc. A number of methods are available to analyze nucleic acids for the presence of a specific sequence, *e.g.*, a disease associated polymorphism. Genomic DNA can be used when large amounts of DNA are available. Alternatively, the region of interest is cloned into a suitable vector and grown in sufficient quantity for analysis. Cells that express the gene provide a source of mRNA, which can be

assayed directly or reverse transcribed into cDNA for analysis. The nucleic acid can be amplified by conventional techniques, *i.e.*, PCR, to provide sufficient amounts for analysis. (Saiki et al., 1988; Sambrook et al., 1989, pp.14.2-14.33). Alternatively, various methods are known in the art that utilize oligonucleotide ligation as a means of detecting polymorphisms (Riley et al., 1990; Delahunty et al., 1996).

[0245] The sample nucleic acid, *e.g.*, an amplified or cloned fragment, is analyzed by one of a number of methods known in the art. The nucleic acid can be sequenced by dideoxy nucleotide sequencing, or other methods, and the sequence of bases compared to a wild-type sequence. Hybridization with the variant sequence can also be used to determine its presence, *e.g.*, by Southern blots, dot blots, etc. The hybridization pattern of a control and variant sequence to an array of oligonucleotide probes immobilized on a solid support, as described in US Pat. No. 5,445,934, or WO 95/35505, can also be used as a means of detecting the presence of variant sequences. Single strand conformational polymorphism (SSCP) analysis, denaturing gradient gel electrophoresis (DGGE), and heteroduplex analysis in gel matrices can detect variation as alterations in electrophoretic mobility resulting from conformational changes created by DNA sequence alterations. Alternatively, where a polymorphism creates or destroys a recognition site for a restriction endonuclease, the sample can be digested with that endonuclease, and the products fractionated according to their size to determine whether the fragment was digested. Fractionation can be performed by gel or capillary electrophoresis, for example with acrylamide or agarose gels.

[0246] Screening for mutations in a gene can be based on the functional or antigenic characteristics of the protein. Protein truncation assays are useful in detecting deletions that might affect the biological activity of the protein. Various immunoassays designed to detect polymorphisms in proteins can be used in screening. Where many diverse genetic mutations lead to a particular disease phenotype, functional protein assays have proven to be effective screening tools. The activity of the encoded protein can be determined by comparison with the wild-type protein.

#### *4. Detecting and Monitoring Polypeptide Presence and Biological Activity*

[0247] The present invention provides methods for detecting the presence and/or biological activity of a subject polypeptide in a biological sample. The assay used will be appropriate to the biological activity of the particular polypeptide. Thus, *e.g.*, where the biological activity is an enzymatic activity, the method will involve contacting the sample with an appropriate substrate, and detecting the product of the enzymatic reaction on the

substrate. Where the biological activity is binding to a second macromolecule, the assay detects protein-protein binding, protein-DNA binding, protein-carbohydrate binding, or protein-lipid binding, as appropriate, using well known assays. Where the biological activity is signal transduction (*e.g.*, transmission of a signal from outside the cell to inside the cell) or transport, an appropriate assay is used, such as measurement of intracellular calcium ion concentration, measurement of membrane conductance changes, or measurement of intracellular potassium ion concentration.

[0248] The present invention also provides methods for detecting the presence or measuring the level of a normal or abnormal polypeptide in a biological sample using a specific antibody. The methods generally comprise contacting the sample with a specific antibody and detecting binding between the antibody and molecules of the sample. Specific antibody binding, when compared to a suitable control, is an indication that a polypeptide of interest is present in the sample. Suitable controls include a sample known not to contain the polypeptide, and a sample contacted with a non-specific antibody, *e.g.*, an anti-idiotypic antibody.

[0249] A variety of methods to detect specific antibody-antigen interactions are known in the art, *e.g.*, standard immunohistological methods, immunoprecipitation, enzyme immunoassay, and radioimmunoassay. The specific antibody can be detectably labeled, either directly or indirectly, as described at length herein, and cells are permeabilized to stain cytoplasmic molecules. Briefly, antibodies are added to a cell sample, and incubated for a period of time sufficient to allow binding to the epitope, usually at least about 10 minutes. The antibody may be labeled with radioisotopes, enzymes, fluorescers, chemiluminescers, or other labels for direct detection.. Alternatively, specific-binding pairs may be used, involving, *e.g.*, a second stage antibody or reagent that is detectably-labeled, as described above. Such reagents and their methods of use are well known in the art

[0250] Alternatively, a biological sample can be brought into contact with an immobilized antibody on a solid support or carrier, such as nitrocellulose, that is capable of immobilizing cells, cell particles, or soluble proteins. The antibody can be attached (coupled) to an insoluble support, such as a polystyrene plate or a bead. After contacting the sample, the support can then be washed with suitable buffers, followed by contacting with a detectably-labeled specific antibody. Detection methods are known in the art and will be chosen as appropriate to the signal emitted by the detectable label. Detection is generally accomplished in comparison to suitable controls, and to appropriate standards.

[0251] The present invention further provides methods for detecting the presence and/or levels of enzymatic activity of a subject polypeptide in a biological sample. The methods generally involve contacting the sample with a substrate that yields a detectable product upon being acted upon by a subject polypeptide, and detecting a product of the enzymatic reaction. Further, polypeptides that are subsets of the complete sequences of the subject proteins may be used to identify and investigate parts of the protein important for function.

[0252] The present invention further includes methods for monitoring activity of a polypeptide through observation of phenotypic changes in a cell containing such polypeptide, such as growth or differentiation, or the ability of such a cell to secrete a molecule that can be detected, such as through chemical methods or through its effect on another cell, such as cell activation.

#### *5. Modulating mRNA and Peptides in Biological Samples*

[0253] The present invention provides screening methods for identifying agents that modulate the level of a mRNA molecule of the invention, agents that modulate the level of a polypeptide of the invention, and agents that modulate the biological activity of a polypeptide of the invention. In some embodiments, the assay is cell-free; in others, it is cell-based. Where the screening assay is a binding assay, one or more of the molecules can be joined to a label, where the label can directly or indirectly provide a detectable signal.

[0254] As discussed above, the invention encompasses endogenous polynucleotides of the invention that encode mRNA and/or polypeptides of interest. Again as discussed previously, the invention also encompasses exogenous polynucleotides that encode mRNA or polypeptides of the invention. For example, the polynucleotide can reside within a recombinant vector which is introduced into the cell. For example, a recombinant vector can comprise an isolated transcriptional regulatory sequence which is associated in nature with a nucleic acid, such as a promoter sequence operably linked to sequences coding for a polypeptide of the invention; or the transcriptional control sequences can be operably linked to coding sequences for a polypeptide fusion protein comprising a polypeptide of the invention fused to a polypeptide that facilitates detection.

[0255] In these embodiments, the candidate agent is combined with a cell possessing a polynucleotide transcriptional regulatory element operably linked to a polypeptide-coding sequence of interest, *e.g.*, a subject cDNA or its genomic component; and determining the

agent's effect on polynucleotide expression, as measured, for example by the level of mRNA, polypeptide, or fusion polypeptide.

[0256] In other embodiments, for example, a recombinant vector can comprise an isolated polynucleotide transcriptional regulatory sequence, such as a promoter sequence, operably linked to a reporter gene (*e.g.*,  $\beta$ -galactosidase, CAT, luciferase, or other gene that can be easily assayed for expression). In these embodiments, the method for identifying an agent that modulates a level of expression of a polynucleotide in a cell comprises combining a candidate agent with a cell comprising a transcriptional regulatory element operably linked to a reporter gene; and determining the effect of said agent on reporter gene expression.

[0257] Known methods of measuring mRNA levels can be used to identify agents that modulate mRNA levels, including, but not limited to, PCR with detectably-labeled primers. Similarly, agents that modulate polypeptide levels can be identified using standard methods for determining polypeptide levels, including, but not limited to an immunoassay such as ELISA with detectably-labeled antibodies.

[0258] A wide variety of cell-based assays can also be used to identify agents that modulate eukaryotic or prokaryotic mRNA and/or polypeptide levels. Examples include transformed cells that over-express a cDNA construct and cells transformed with a polynucleotide of interest associated with an endogenously-associated promoter operably linked to a reporter gene. A control sample would comprise, for example, the same cell lacking the candidate agent. Expression levels are measured and compared in the test and control samples.

[0259] The cells used in the assay are usually mammalian cells, including, but not limited to, rodent cells and human cells. The cells can be primary cell cultures or can be immortalized cell lines. Cell-based assays generally comprise the steps of contacting the cell with a test agent, forming a test sample, and, after a suitable time, assessing the agent's effect on macromolecule expression. That is, the mammalian cell line is transformed or transfected with a construct that results in expression of the polynucleotide, the cell is contacted with a test agent, and then mRNA or polypeptide levels are detected and measured using conventional assays.

[0260] A suitable period of time for contacting the agent with the cell can be determined empirically, and is generally a time sufficient to allow entry of the agent into the cell and to allow the agent to have a measurable effect on subject mRNA and/or polypeptide levels. Generally, a suitable time is between about 10 minutes and about 24 hours, including

about 1 to about 8 hours. Alternatively, incubation periods may be between about 0.1 and about 1 hour, selected for example for optimum activity or to facilitate rapid high-throughput screening. Where the polypeptide is expressed on the cell surface, however, a shorter length of time may be sufficient. Incubations are performed at any suitable temperature, *i.e.*, between about 4°C and about 40°C. The contact and incubation steps can be followed by a washing step to remove unbound components, *i.e.*, a label that would give rise to a background signal during subsequent detection of specifically-bound complexes.

[0261] A variety of assay configurations and protocols are known in the art. For example, one of the components can be bound to a solid support, and the remaining components contacted with the support bound component. Remaining components may be added at different times or at substantially the same time. Further, where the interacting protein is a second subject protein, the effect of the test agent on binding can be determined by determining the effect on multimerization of the subject protein.

[0262] The present invention further provides methods of identifying agents that modulate a biological activity of a polypeptide of the invention. The method generally comprises contacting a test agent with a sample containing a subject polypeptide and assaying a biological activity of the subject polypeptide in the presence of the test agent. An increase or a decrease in the assayed biological activity in comparison to the activity in a suitable control (*e.g.*, a sample comprising a subject polypeptide in the absence of the test agent) is an indication that the substance modulates a biological activity of the subject polypeptide. The mixture of components is added in any order that provides for the requisite interaction..

[0263] External and internal processes that can affect modulation of a macromolecule of the invention include, but are not limited to, infection of a cell by a microorganism, including, but not limited to, a bacterium (*e.g.*, *Mycobacterium* spp., *Shigella*, or *Chlamydia*), a protozoan (*e.g.*, *Trypanosoma* spp., *Plasmodium* spp., or *Toxoplasma* spp.), a fungus, a yeast (*e.g.*, *Candida* spp.), or a virus (including viruses that infect mammalian cells, such as human immunodeficiency virus, foot and mouth disease virus, Epstein-Barr virus, and viruses that infect plant cells); change in pH of the medium in which a cell is maintained or a change in internal pH; excessive heat relative to the normal range for the cell or the multicellular organism; excessive cold relative to the normal range for the cell or the multicellular organism; an effector molecule such as a hormone, a cytokine, a chemokine, a neurotransmitter; an ingested or applied drug; a ligand for a cell-surface receptor; a ligand for

a receptor that exists internally in a cell, *e.g.*, a nuclear receptor; hypoxia; light; dark; sleep patterns; electrical charge; ion concentration of the medium in which a cell is maintained or an internal ion concentration, exemplary ions including sodium ions, potassium ions, chloride ions, calcium ions, and the like; presence or absence of a nutrient; metal ions; a transcription factor; mitogens, including, but not limited to, lipopolysaccharide (LPS), pokeweed mitogen; antigens; a tumor suppressor; and cell-cell contact and must be taken into consideration in the screening assay.

[0264] A variety of other reagents can be included in the screening assay. These include salts, neutral proteins, *e.g.*, albumin, detergents, and other compounds that facilitate optimal binding and/or reduce non-specific or background interactions. Reagents that improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, or anti-microbial agents, etc., can be used.

[0265] Accordingly, the present invention provides a method for identifying an agent, particularly a biologically active agent that modulates the level of expression of a nucleic acid in a cell, the method comprising: combining a candidate agent to be tested with a cell comprising a nucleic acid that encodes a polypeptide, and determining the agent's effect on polypeptide expression.

[0266] Some embodiments will detect agents that decrease the biological activity of a molecule of the invention. Maximal inhibition of the activity is not always necessary, or even desired, in every instance to achieve a therapeutic effect. Agents that decrease a biological activity can find use in treating disorders associated with the biological activity of the molecule. Alternatively, some embodiments will detect agents that increase a biological activity. Agents that increase a biological activity of a molecule of the invention can find use in treating disorders associated with a deficiency in the biological activity. Agents that increase or decrease a biological activity of a molecule of the invention can be selected for further study, and assessed for physiological attributes, *i.e.*, cellular availability, cytotoxicity, or biocompatibility, and optimized as required. For example, a candidate agent is assessed for any cytotoxic activity it may exhibit toward the cell used in the assay using well-known assays, such as trypan blue dye exclusion, an MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2 H-tetrazolium bromide]) assay, and the like.

[0267] A variety of different candidate agents can be screened by the above methods. Candidate agents encompass numerous chemical classes, as described above.

[0268] Candidate agents are obtained from a wide variety of sources including libraries of synthetic or natural compounds. Numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides and oligopeptides. For example, random peptide libraries obtained by yeast two-hybrid screens (Xu et al., 1997), phage libraries (Hoogenboom et al., 1998), or chemically generated libraries. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced, including antibodies produced upon immunization of an animal with subject polypeptides, or fragments thereof, or with the encoding polynucleotides. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means, and can be used to produce combinatorial libraries. Further, known pharmacological agents can be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, and amidification, etc, to produce structural analogs.

#### 6. Kits

[0269] The present invention provides methods for diagnosing disease states based on the detected presence and/or level of polynucleotide or polypeptide in a biological sample, and/or the detected presence and/or level of biological activity of the polynucleotide or polypeptide. These detection methods can be provided as part of a kit. Thus, the invention further provides kits for detecting the presence and/or a level of a polynucleotide or polypeptide in a biological sample and/or or the detected presence and/or level of biological activity of the polynucleotide or polypeptide. Procedures using these kits can be performed by clinical laboratories, experimental laboratories, medical practitioners, or private individuals.

[0270] The kits of the invention will comprise a molecule of the invention. The kits for detecting a polynucleotide will also comprise a moiety that specifically hybridizes to a polynucleotide of the invention. The polynucleotide molecule can be of any length. For example, it can comprise a polynucleotide of at least 6, at least 7, at least 8, or at least 9 contiguous nucleotides of a molecule of the invention. Kits of the invention for detecting a subject polypeptide will comprise a moiety that specifically binds to a polypeptide of the invention; the moiety includes, but is not limited to, a polypeptide-specific antibody.



[0271] The kits are useful in diagnostic applications. For example, the kit is useful to determine whether a given DNA sample isolated from an individual comprises an expressed nucleic acid, a polymorphism, or other variant.

[0272] Kits for detecting polynucleotides comprise a pair of nucleic acids in a suitable storage medium, *e.g.*, a buffered solution, in a suitable container. The pair of isolated nucleic acid molecules serve as primers in an amplification reaction (*e.g.*, a polymerase chain reaction). The kit can further include additional buffers, reagents for polymerase chain reaction (*e.g.*, deoxynucleotide triphosphates (dNTP), a thermostable DNA polymerase, a solution containing  $Mg^{2+}$  ions (*e.g.*,  $MgCl_2$ ), and other components well known to those skilled in the art for carrying out a polymerase chain reaction). The kit can further include instructions for use, which may be provided in a variety of forms, *e.g.*, printed information, or compact disc, and the like. The kit may further include reagents necessary to extract DNA from a biological sample and reagents for generating a cDNA copy of an mRNA. The kit may optionally provide additional useful components, including, but not limited to, buffers, developing reagents, labels, reacting surfaces, means for detections, control samples, standards, and interpretive information.

[0273] In some embodiments, a kit of the invention for detecting a polynucleotide, such as an mRNA encoding a polypeptide, comprises a pair of nucleic acids that function as "forward" and "reverse" primers that specifically amplify a cDNA copy of the mRNA. The "forward" and "reverse" primers are provided as a pair of isolated nucleic acid molecules, each from about 10 to about 200 nucleotides in length, the first nucleic acid molecule of the pair comprising a sequence of at least about 10 contiguous nucleotides having 100% sequence identity to a nucleic acid sequence shown in SEQ. ID. NOS. 1-187 and 375-484, and the second nucleic acid molecule of the pair comprising a sequence of at least about 10 contiguous nucleotides having 100% sequence identity to the reverse complement of a nucleic acid sequence shown in SEQ. ID. NOS. 1-187 and 375-484, wherein the sequence of the second nucleic acid molecule is located 3' of the nucleic acid sequence of the first nucleic acid molecule. The primer nucleic acids are prepared using any known method, *e.g.*, automated synthesis. In some embodiments, one or both members of the pair of nucleic acid molecules comprise a detectable label.

[0274] Where the kit provides for polypeptide detection, it can include one or more specific antibodies. In some embodiments, the antibody specific to the polypeptide is detectably labeled. In other embodiments, the antibody specific to the polypeptide is not

labeled; instead, a second, detectably-labeled antibody is provided that binds to the specific antibody. The kit may further include blocking reagents, buffers, and reagents for developing and/or detecting the detectable marker. The kit may further include instructions for use, controls, and interpretive information.

[0275] Where the kit provides for detecting enzymatic activity, it includes a substrate that provides for a detectable product when acted upon by a polypeptide of interest. The kit may further include reagents necessary to detect and develop the detectable marker.

[0276] The present invention provides for kits with unit doses of an active agent. These agents are described in more detail below. In some embodiments, the agent is provided in oral or injectable doses. Such kits will comprise containers containing the unit doses and an informational package insert describing the use and attendant benefits of the drugs in treating a condition of interest.

#### *Stem Cells*

[0277] Stem cells are pluripotent or multipotent cells that generate maturing cells in multiple differentiation lineages. Embryonic stem cells are pluripotent; they can differentiate into any of the cells present in the adult. Multipotent cells have the ability to differentiate into more than one cell type. Organ-specific stem cells are multipotent; they can differentiate into any of the cells of the organ they inhabit.

[0278] When they divide *in vivo*, both pluripotent and multipotent stem cells can maintain their pluripotency or multipotency while giving rise to differentiated progeny. Thus, stem cells can produce replicas of themselves which are pluri- or multipotent, and are also able to differentiate into lineage-restricted committed progenitor cells. For example, hematopoietic stem cells, which are multipotent cells specifically able to form blood cells, can divide to produce replicate hematopoietic stem cells. They can also divide to produce more highly differentiated cells, which are precursors of blood cells. The precursors differentiate, sometimes through several generations of cells, into blood cells. A hematopoietic stem cell can also divide into a cell with the capacity to form, for example, a relatively undifferentiated cell that is committed to differentiate into, *i.e.*, granulocytes, erythrocytes, or another type of blood cell.

[0279] Stem cells can also reproduce and differentiate *in vitro*. Embryonic stem cells have been directed to differentiate into cardiac muscle cells *in vitro* and, alternatively, into early progenitors of neural stem cells, and then into mature neurons and glial cells *in vitro* (Trounson, 2002).

[0280] The study of stem cells has led to therapy for treating cancer in humans (Slavin et al., 2001). Stem cell therapy offers several advantages over traditional cancer therapies (Weissman, 2000). One advantage of stem cell therapy exists when used in conjunction with radiation therapy. In radiation therapy for cancer, the dose of radiation necessary to kill the cancer cells in an organ can also be sufficient to destroy the healthy cells of the organ. In combined stem cell and radiation therapy, an organ is first treated with sufficient radiation to destroy all of the cancer cells and most or all of the healthy cells, but then stem cells are infused to repopulate the organ. In the ensuing weeks, as the cancer cells and healthy cells die, the stem cells replace the healthy cells. Another advantage of this approach, compared to heterologous organ transplants, is that there is no risk of rejection, since stem cells do not provoke an immune response. A further advantage is that stem cells are inherently programmed to regulate their numbers and differentiation status, *i.e.*, once provided to the patient, the necessary number will differentiate, and the rest will remain undifferentiated (Weissman, 2000).

[0281] Stem cell therapy is also effective in treating autoimmune disease in humans. For example, immunosuppression in conjunction with stem-cell transplantation has induced remission in patients with refractory, severe rheumatic autoimmune disease (Van Laar and Tyndall, 2003). Patients with rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis, and juvenile idiopathic arthritis have benefited from stem cell transplants (Van Laar and Tyndall, 2003).

[0282] Preclinical studies also suggest the potential of stem cell transplantation for the treatment of neural and muscular injuries and diseases, including those of the central nervous system, peripheral nervous system, and skeletal, cardiac and smooth muscle (Deasy and Huard, 2002). Stem cells transplanted into the bone marrow of mice migrate to the site of injured muscle and differentiate into new muscle cells. Thus, patients with myasthenia gravis, muscular dystrophies, amyotrophic lateral sclerosis, congestive heart failure, Parkinson's disease, and Alzheimer's disease may benefit from stem cell therapy (Henningson, 2003).

[0283] In addition to therapeutic uses, research using stem cells can provide useful information about normal stem cell function and the pathogenesis of disease. Stem cells derived from a patient with a genetic disease can provide a tool for studying that disease. To derive these stem cells, a somatic cell, *i.e.*, a cell that is not in the oocyte or spermatocyte lineage, is donated by the patient, and the nucleus is removed and transferred to an

unfertilized human oocyte. This nuclear transplant procedure produces, at the blastocyst stage of development, embryonic stem cells with the same set of genes as the patient with the genetic disease. Studying these cells, and their progeny *in vitro*, permits analysis of a specific model of the disease. For example, placing stem cells derived from a patient with a genetic disease under the control of various stem cell regulatory factors can elicit abnormal responses from the affected stem cells compared to stem cells derived from a healthy individual's somatic nucleus.

[0284] A specific type of stem cell, mesenchymal stem cells (MSC) are adult pluripotent stem cells present *in vivo* in the bone marrow, and able to travel to and populate different sites in the body. MSCs derive from a common progenitor cell, the multipotent adult progenitor cell (MAPC), that gives rise to other lineages as different as endothelium, endoderm, and ectoderm. MSCs self-renew by proliferation while maintaining their stem cell phenotype and give rise to the differentiated stromal cells which belong to the osteogenic, chondrogenic, adipogenic, myogenic and fibroblastic lineages. They are capable of differentiating into a number of different cell types, including osteocytes, chondrocytes, adipocytes, myocytes, and fibroblasts (Van Damme et al., 2002).

[0285] MSCs can be identified by their expression of thy-1 (CD90), endoglin (CD105), vascular cell adhesion molecule 1 (VCAM-1) (CD106), and hyaluronate receptor (CD44). MSC potential can be determined by the colony-forming unit fibroblast assay (CFU-F) (Jorgensen et al., 2003). They can be expanded in culture without losing their phenotype or multilineage potential (Jorgensen et al., 2003).

[0286] MSCs are present in tissues involved in autoimmune diseases. Since MSCs can target the affected organs in autoimmune diseases, they can be used as a vehicle to express therapeutic proteins (Jorgensen et al., 2003).

[0287] MSCs have been demonstrated to be present in mesenchymal-derived tissue, such as injured synovium in the presence of chronic inflammation (Jorgensen et al., 2003).

[0288] MSCs themselves display immunological properties; these properties can enhance their therapeutic potential (Jorgensen et al., 2003). For example, they suppress T-cell responses (Jorgensen et al., 2003). Systemically infused autologous MSCs delayed donor cell rejection from a control value of 7 days to a treated value of 11 days in a murine skin graft model (Jorgensen et al., 2003).

[0289] Thus, MSCs can be used in different therapeutic strategies, either as immunosuppressive agents, or as genetically engineered vehicles for therapeutics. Since

MSCs are not rejected, and they travel to injured mesenchymal tissues, they can be used as a means of transportation to deliver therapeutic proteins to a targeted area. The capacity of MSCs to adhere to matrix components favors their preferential homing to bone, lung, and cartilage when injected intravenously (Jorgensen et al., 2003). They also home preferentially to the bone marrow. Cell mediated therapy offers the advantage that the autologous MCS can be used in treatment.

[0290] MSCs have been genetically modified to express anti-inflammatory cytokines. They have been demonstrated to secrete several therapeutic heterologous proteins, such as human erythropoietin and factor IX (Jorgensen et al., 2003). Rat mesenchymal stem cells have been genetically engineered using *ex vivo* retroviral transduction to overexpress the prosurvival gene Akt1, a protein kinase that is the product of an oncogene (Mangi et al., 2003).

[0291] The exogenous proteins expressed by genetically altered mesenchymal stem cells can exert their therapeutic effect by targeting cells present in the environment of the stem cell. The altered stem cells can also respond to the exogenous protein that it secretes. The ability of these altered cells to respond to the transgene, as well as other local signals *in vivo* enables the development of gene therapy strategies that exploit the benefits of particular therapeutic proteins. For example, mesenchymal stem cells that express bone-promoting osteogenic factors can express factors that promote bone growth and can also respond, differentiate, and participate in the bone formation process (Turgeman et al., 2002). Mesenchymal stem cells have also been used to repair defects in cartilage and ligaments (Pend and Huard, 2003), and express a furin-cleavable insulin gene (Sasaki et al., 2003).

#### *Therapeutic Compositions*

[0292] The invention further provides agents identified using a screening assay of the invention, and compositions comprising the agents, subject polypeptides, subject polynucleotides, recombinant vectors, and/or host cells, including pharmaceutical compositions for therapeutic administration. The subject compositions can be formulated using well-known reagents and methods. These compositions can include a buffer, which is selected according to the desired use of the agent, polypeptide, polynucleotide, recombinant vector, or host cell, and can also include other substances appropriate to the intended use. Those skilled in the art can readily select an appropriate buffer, a wide variety of which are known in the art, suitable for an intended use.

### *1. Excipients and Formulations*

[0293] In some embodiments, compositions are provided in formulation with pharmaceutically acceptable excipients, a wide variety of which are known in the art (Gennaro, 2000; Ansel et al., 1999; Kibbe et al., 2000). Pharmaceutically acceptable excipients, such as vehicles, adjuvants, carriers or diluents, are readily available to the public. Moreover, pharmaceutically acceptable auxiliary substances, such as pH adjusting and buffering agents, tonicity adjusting agents, stabilizers, wetting agents and the like, are readily available to the public.

[0294] In pharmaceutical dosage forms, the compositions of the invention can be administered in the form of their pharmaceutically acceptable salts, or they can also be used alone or in appropriate association, as well as in combination, with other pharmaceutically active compounds. The subject compositions are formulated in accordance to the mode of potential administration. Administration of the agents can be achieved in various ways, including oral, buccal, nasal, rectal, parenteral, intraperitoneal, intradermal, transdermal, subcutaneous, intravenous, intra-arterial, intracardiac, intraventricular, intracranial, intratracheal, and intrathecal administration, etc., or otherwise by implantation or inhalation. Thus, the subject compositions can be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants and aerosols. The following methods and excipients are merely exemplary and are in no way limiting.

[0295] For oral preparations, the agents, polynucleotides, and polypeptides can be used alone or in combination with appropriate additives to make tablets, powders, granules or capsules, for example, with conventional additives, such as lactose, mannitol, corn starch, or potato starch; with binders, such as crystalline cellulose, cellulose derivatives, acacia, corn starch, or gelatins; with disintegrators, such as corn starch, potato starch, or sodium carboxymethylcellulose; with lubricants, such as talc or magnesium stearate; and if desired, with diluents, buffering agents, moistening agents, preservatives, and flavoring agents.

[0296] Suitable excipient vehicles are, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vehicle can contain minor amounts of auxiliary substances such as wetting or emulsifying agents or pH buffering agents. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in the art (Remington, 1985). The composition or formulation to be

administered will, in any event, contain a quantity of the agent adequate to achieve the desired state in the subject being treated.

[0297] The agents, polynucleotides, and polypeptides can be formulated into preparations for injection by dissolving, suspending or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives. Other formulations for oral or parenteral delivery can also be used, as conventional in the art

[0298] The agents, polynucleotides, and polypeptides can be utilized in aerosol formulation to be administered via inhalation. The compounds of the present invention can be formulated into pressurized acceptable propellants such as dichlorodifluoromethane, propane, nitrogen, and the like. Further, the agent, polynucleotides, or polypeptide composition may be converted to powder form for administration intranasally or by inhalation, as conventional in the art.

[0299] Furthermore, the agents can be made into suppositories by mixing with a variety of bases such as emulsifying bases or water-soluble bases. The compounds of the present invention can be administered rectally via a suppository. The suppository can include vehicles such as cocoa butter, carbowaxes and polyethylene glycols, which melt at body temperature, yet are solidified at room temperature.

[0300] A polynucleotide, polypeptide, or other modulator, can also be introduced into tissues or host cells by other routes, such as viral infection, microinjection, or vesicle fusion. For example, expression vectors can be used to introduce nucleic acid compositions into a cell as described above. Further, jet injection can be used for intramuscular administration (Furth et al., 1992). The DNA can be coated onto gold microparticles, and delivered intradermally by a particle bombardment device, or "gene gun" as described in the literature (Tang et al., 1992), where gold microprojectiles are coated with the DNA, then bombarded into skin cells.

[0301] Unit dosage forms for oral or rectal administration such as syrups, elixirs, and suspensions can be provided wherein each dosage unit, for example, teaspoonful, tablespoonful, tablet, or suppository, contains a predetermined amount of the composition containing one or more agents. Similarly, unit dosage forms for injection or intravenous

administration can comprise the agent(s) in a composition as a solution in sterile water, normal saline or another pharmaceutically acceptable carrier.

## 2. *Active Agents (or Modulators)*

[0302] The nucleic acid, polypeptide, and modulator compositions of the subject invention find use as therapeutic agents in situations where one wishes to modulate an activity of a subject polypeptide in a host, particularly the activity of the subject polypeptides, or to provide or inhibit the activity at a particular anatomical site. Thus, the compositions are useful in treating disorders associated with an activity of a subject polypeptide. The following provides further details of active agents of the present invention.

### *a) Antisense Oligonucleotides*

[0303] In certain embodiments of the invention, the active agent is an agent that modulates, and generally decreases or down regulates, the expression of a gene encoding a target protein in a host, *i.e.*, antisense molecules. Anti-sense reagents include antisense oligonucleotides (ODN), *i.e.*, synthetic ODN having chemical modifications from native nucleic acids, or nucleic acid constructs that express such anti-sense molecules as RNA. The antisense sequence is complementary to the mRNA of the targeted gene, and inhibits expression of the targeted gene products. Antisense molecules inhibit gene expression through various mechanisms, *e.g.*, by reducing the amount of mRNA available for translation, through activation of RNase H, or steric hindrance. One or a combination of antisense molecules can be administered, where a combination can comprise multiple different sequences.

[0304] Antisense molecules can be produced by expression of all or a part of the target gene sequence in an appropriate vector, where the transcriptional initiation is oriented such that an antisense strand is produced as an RNA molecule. Alternatively, the antisense molecule is a synthetic oligonucleotide. Antisense oligonucleotides can be chemically synthesized by methods known in the art (Wagner et al., 1993; Milligan et al., 1993). Oligonucleotides can be chemically modified from the native phosphodiester structure to increase their intracellular stability and binding affinity, for example, as described in detail above. Antisense oligonucleotides will generally be at least about 7, at least about 12, or at least about 20 nucleotides in length, and not more than about 500, not more than about 50, or not more than about 35 nucleotides in length, where the length is governed by efficiency of inhibition, and specificity, including absence of cross-reactivity, and the like. Short



oligonucleotides, of from about 7 to about 8 bases in length, can be strong and selective inhibitors of gene expression (Wagner et al., 1996).

[0305] A specific region or regions of the endogenous sense strand mRNA sequence is chosen to be complemented by the antisense sequence. Selection of a specific sequence for the oligonucleotide can use an empirical method, where several candidate sequences are assayed for inhibition of expression of the target gene in an *in vitro* or animal model. A combination of sequences can also be used, where several regions of the mRNA sequence are selected for antisense complementation.

[0306] As an alternative to anti-sense inhibitors, catalytic nucleic acid compounds, *e.g.*, ribozymes, or anti-sense conjugates can be used to inhibit gene expression. Ribozymes can be synthesized *in vitro* and administered to the patient, or can be encoded in an expression vector, from which the ribozyme is synthesized in the targeted cell (WO 9523225; Beigelman et al., 1995). Examples of oligonucleotides with catalytic activity are described in WO 9506764. Conjugates of anti-sense ODN with a metal complex, *e.g.*, terpyridyl Cu(II), capable of mediating mRNA hydrolysis are described in Bashkin *et al.*, 1995.

#### *b) Interfering RNA*

[0307] In some embodiments, the active agent is an interfering RNA (RNAi), including dsRNAi. RNA interference provides a method of silencing eukaryotic genes. Double stranded RNA can induce the homology-dependent degradation of its cognate mRNA in *C. elegans*, fungi, plants, *Drosophila*, and mammals (Gaudilliere et al., 2002). Use of RNAi to reduce a level of a particular mRNA and/or protein is based on the interfering properties of double-stranded RNA derived from the coding regions of a gene. The technique reduces the time between identifying an interesting gene sequence and understanding its function, and thus is an efficient high-throughput method for disrupting gene function (O'Neil, 2001). RNAi can also help identify the biochemical mode of action of a drug and to identify other genes encoding products that can respond or interact with specific compounds.

[0308] In one embodiment of the invention, complementary sense and antisense RNAs derived from a substantial portion of the subject polynucleotide are synthesized *in vitro*. The resulting sense and antisense RNAs are annealed in an injection buffer, and the double-stranded RNA injected or otherwise introduced into the subject, *i.e.*, in food or by immersion in buffer containing the RNA (Gaudilliere et al., 2002; O'Neil et al., 2001; WO99/32619). In another embodiment, dsRNA derived from a gene of the present invention is generated *in vivo* by simultaneously expressing both sense and antisense RNA from

appropriately positioned promoters operably linked to coding sequences in both sense and antisense orientations.

*c) Peptides and Modified Peptides*

[0309] In some embodiments of the present invention, the active agent is a peptide. Suitable peptides include peptides of from about 3 amino acids to about 50, from about 5 to about 30, or from about 10 to about 25 amino acids in length. In some embodiments, a peptide has a sequence of from about 3 amino acids to about 50, from about 5 to about 30, or from about 10 to about 25 amino acids of corresponding naturally-occurring protein. In some embodiments, a peptide exhibits one or more of the following activities: inhibits binding of a subject polypeptide to an interacting protein or other molecule; inhibits subject polypeptide binding to a second polypeptide molecule; inhibits a signal transduction activity of a subject polypeptide; inhibits an enzymatic activity of a subject polypeptide; or inhibits a DNA binding activity of a subject polypeptide.

[0310] Peptides can include naturally-occurring and non-naturally occurring amino acids. Peptides can comprise D-amino acids, a combination of D- and L-amino acids, and various "designer" amino acids (*e.g.*,  $\beta$ -methyl amino acids, C $\alpha$ -methyl amino acids, and N $\alpha$ -methyl amino acids, etc.) to convey special properties. Additionally, peptides can be cyclic. Peptides can include non-classical amino acids in order to introduce particular conformational motifs. Any known non-classical amino acid can be used. Non-classical amino acids include, but are not limited to, 1,2,3,4-tetrahydroisoquinoline-3-carboxylate; (2S,3S)-methylphenylalanine, (2S,3R)-methyl-phenylalanine, (2R,3S)-methyl-phenylalanine and (2R,3R)-methyl-phenylalanine; 2-aminotetrahydronaphthalene-2-carboxylic acid; hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate;  $\beta$ -carboline (D and L); HIC (histidine isoquinoline carboxylic acid); and HIC (histidine cyclic urea). Amino acid analogs and peptidomimetics can be incorporated into a peptide to induce or favor specific secondary structures, including, but not limited to, LL-Acp (LL-3-amino-2-propenidone-6-carboxylic acid), a  $\beta$ -turn inducing dipeptide analog;  $\beta$ -sheet inducing analogs;  $\beta$ -turn inducing analogs;  $\alpha$ -helix inducing analogs;  $\gamma$ -turn inducing analogs; Gly-Ala turn analogs; amide bond isostere; or tretrazol, and the like.

[0311] A peptide can be a depsipeptide, which can be linear or cyclic (Kuisle et al., 1999). Linear depsipeptides can comprise rings formed through S-S bridges, or through an hydroxy or a mercapto group of an hydroxy-, or mercapto-amino acid and the carboxyl group of another amino- or hydroxy-acid but do not comprise rings formed only through peptide or

ester links derived from hydroxy carboxylic acids. Cyclic depsipeptides contain at least one ring formed only through peptide or ester links, derived from hydroxy carboxylic acids.

[0312] Peptides can be cyclic or bicyclic. For example, the C-terminal carboxyl group or a C-terminal ester can be induced to cyclize by internal displacement of the -OH or the ester (-OR) of the carboxyl group or ester respectively with the N-terminal amino group to form a cyclic peptide. For example, after synthesis and cleavage to give the peptide acid, the free acid is converted to an activated ester by an appropriate carboxyl group activator such as dicyclohexylcarbodiimide (DCC) in solution, for example, in methylene chloride (CH<sub>2</sub>Cl<sub>2</sub>), dimethyl formamide (DMF) mixtures. The cyclic peptide is then formed by internal displacement of the activated ester with the N-terminal amine. Internal cyclization as opposed to polymerization can be enhanced by use of very dilute solutions. Methods for making cyclic peptides are well known in the art.

[0313] The term "bicyclic" refers to a peptide with two ring closures formed by covalent linkages between amino acids. A covalent linkage between two nonadjacent amino acids constitutes a ring closure, as does a second covalent linkage between a pair of adjacent amino acids which are already linked by a covalent peptide linkage. The covalent linkages forming the ring closures can be amide linkages, *i.e.*, the linkage formed between a free amino on one amino acid and a free carboxyl of a second amino acid, or linkages formed between the side chains or "R" groups of amino acids in the peptides. Thus, bicyclic peptides can be "true" bicyclic peptides, *i.e.*, peptides cyclized by the formation of a peptide bond between the N-terminus and the C-terminus of the peptide, or they can be "depsi-bicyclic" peptides, *i.e.*, peptides in which the terminal amino acids are covalently linked through their side chain moieties.

[0314] A desamino or descarboxy residue can be incorporated at the terminal ends of the peptide, so that there is no terminal amino or carboxyl group, to decrease susceptibility to proteases or to restrict conformation. C-terminal functional groups include amide, amide lower alkyl, amide di (lower alkyl), lower alkoxy, hydroxy, and carboxy, and the lower ester derivatives thereof, and the pharmaceutically acceptable salts thereof.

[0315] In addition to the foregoing N-terminal and C-terminal modifications, a peptide or peptidomimetic can be modified with or covalently coupled to one or more of a variety of hydrophilic polymers to increase solubility and circulation half-life of the peptide. Suitable nonproteinaceous hydrophilic polymers for coupling to a peptide include, but are not limited to, polyalkylethers as exemplified by polyethylene glycol and polypropylene glycol,

polylactic acid, polyglycolic acid, polyoxyalkenes, polyvinylalcohol, polyvinylpyrrolidone, cellulose and cellulose derivatives, dextran, and dextran derivatives. Generally, such hydrophilic polymers have an average molecular weight ranging from about 500 to about 100,000 daltons, from about 2,000 to about 40,000 daltons, or from about 5,000 to about 20,000 daltons. The peptide can be derivatized with or coupled to such polymers using any of the methods set forth in Zallipsky, 1995; Monfardini et al., 1995; U.S. Pat. Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192; 4,179,337, or WO 95/34326.

*d) Antibodies*

[0316] The invention provides antibodies that specifically recognize a particular polypeptide. Antibodies are obtained by immunizing a host animal with peptides, polynucleotides encoding polypeptides, or cells, each comprising all or a portion of the target protein ("immunogen"). Suitable host animals include rodents (*e.g.*, mouse, rat, guinea pig, hamster), cattle (*e.g.*, sheep, pig, cow, horse, goat), cat, dog, chicken, primate, monkey, and rabbit. The origin of the protein immunogen can be any species, including mouse, human, rat, monkey, avian, insect, reptile, or crustacean. The host animal will generally be a different species than the immunogen, *e.g.*, a human protein used to immunize mice. Methods of antibody production are well known in the art (Howard and Bethell, 2000; Harlow et al., 1998; Harlow and Lane, 1988).

[0317] The immunogen can comprise the complete protein, or fragments and derivatives thereof, or proteins expressed on cell surfaces. Immunogens comprise all or a part of one of the subject proteins, where these amino acids contain post-translational modifications, such as glycosylation, found on the native target protein. Immunogens comprising protein extracellular domains are produced in a variety of ways known in the art, *e.g.*, expression of cloned genes using conventional recombinant methods, or isolation from tumor cell culture supernatants, etc. The immunogen can also be expressed *in vivo* from a polynucleotide encoding the immunogenic peptide introduced into the host animal.

[0318] Polyclonal antibodies are prepared by conventional techniques. These include immunizing the host animal *in vivo* with the target protein (or immunogen) in substantially pure form, for example, comprising less than about 1% contaminant. The immunogen can comprise the complete target protein, fragments, or derivatives thereof. To increase the immune response of the host animal, the target protein can be combined with an adjuvant; suitable adjuvants include alum, dextran, sulfate, large polymeric anions, and oil & water emulsions, *e.g.*, Freund's adjuvant (complete or incomplete). The target protein can also be

conjugated to synthetic carrier proteins or synthetic antigens. The target protein is administered to the host, usually intradermally, with an initial dosage followed by one or more, usually at least two, additional booster dosages. Following immunization, blood from the host will be collected, followed by separation of the serum from blood cells. The immunoglobulin present in the resultant antiserum can be further fractionated using known methods, such as ammonium salt fractionation, or DEAE chromatography and the like.

[0319] The method of producing polyclonal antibodies can be varied in some embodiments of the present invention. For example, instead of using a single substantially isolated polypeptide as an immunogen, one may inject a number of different immunogens into one animal for simultaneous production of a variety of antibodies. In addition to protein immunogens, the immunogens can be nucleic acids (*e.g.*, in the form of plasmids or vectors) that encode the proteins, with facilitating agents, such as liposomes, microspheres, etc, or without such agents, such as "naked" DNA.

[0320] Antibodies can also be prepared using a library approach. Briefly, mRNA is extracted from the spleens of immunized animals to isolate antibody-encoding sequences. The extracted mRNA may be used to make cDNA libraries. Such a cDNA library may be normalized and subtracted in a manner conventional in the art, for example, to subtract out cDNA hybridizing to mRNA of non-immunized animals. The remaining cDNA may be used to create proteins and for selection of antibody molecules or fragments that specifically bind to the immunogen. The cDNA clones of interest, or fragments thereof, can be introduced into an *in vitro* expression system to produce the desired antibodies, as described herein.

[0321] In a further embodiment, polyclonal antibodies can be prepared using phage display libraries, conventional in the art. In this method, a collection of bacteriophages displaying antibody properties on their surfaces are made to contact subject polypeptides, or fragments thereof. Bacteriophages displaying antibody properties that specifically recognize the subject polypeptides are selected, amplified, for example, in *E. coli*, and harvested. Such a method typically produces single chain antibodies

[0322] Monoclonal antibodies are also produced by conventional techniques, such as fusing an antibody-producing plasma cell with an immortal cell to produce hybridomas. Suitable animals will be used, *e.g.*, to raise antibodies against a mouse polypeptide of the invention, the host animal will generally be a hamster, guinea pig, goat, chicken, or rabbit, and the like. Generally, the spleen and/or lymph nodes of an immunized host animal provide the source of plasma cells, which are immortalized by fusion with myeloma cells to produce

hybridoma cells. Culture supernatants from individual hybridomas are screened using standard techniques to identify clones producing antibodies with the desired specificity. The antibody can be purified from the hybridoma cell supernatants or from ascites fluid present in the host by conventional techniques, *e.g.*, affinity chromatography using antigen, *e.g.*, the subject protein, bound to an insoluble support, *i.e.*, protein A sepharose, etc.

[0323] The antibody can be produced as a single chain, instead of the normal multimeric structure of the immunoglobulin molecule. Single chain antibodies have been previously described (*i.e.*, Jost et al., 1994). DNA sequences encoding parts of the immunoglobulin, for example, the variable region of the heavy chain and the variable region of the light chain are ligated to a spacer, such as one encoding at least about four small neutral amino acids, *i.e.*, glycine or serine. The protein encoded by this fusion allows the assembly of a functional variable region that retains the specificity and affinity of the original antibody.

[0324] The invention also provides intrabodies that are intracellularly expressed single-chain antibody molecules designed to specifically bind and inactivate target molecules inside cells. Intrabodies have been used in cell assays and in whole organisms (Chen et al., 1994; Hassanzadeh et al., 1998). Inducible expression vectors can be constructed with intrabodies that react specifically with a protein of the invention. These vectors can be introduced into host cells and model organisms.

[0325] The invention also provides "artificial" antibodies, *e.g.*, antibodies and antibody fragments produced and selected *in vitro*. In some embodiments, these antibodies are displayed on the surface of a bacteriophage or other viral particle, as described above. In other embodiments, artificial antibodies are present as fusion proteins with a viral or bacteriophage structural protein, including, but not limited to, M13 gene III protein. Methods of producing such artificial antibodies are well known in the art (U.S. Patent Nos. 5,516,637; 5,223,409; 5,658,727; 5,667,988; 5,498,538; 5,403,484; 5,571,698; and 5,625,033). The artificial antibodies, selected for example, on the basis of phage binding to selected antigens, can be fused to a Fc fragment of an immunoglobulin for use as a therapeutic, as described, for example, in US 5,116,964 or WO 99/61630. Antibodies of the invention can be used to modulate biological activity of cells, either directly or indirectly. A subject antibody can modulate the activity of a target cell, with which it has primary interaction, or it can modulate the activity of other cells by exerting secondary effects, *i.e.*, when the primary targets interact or communicate with other cells. The antibodies of the invention can be administered to

mammals, and the present invention includes such administration, particularly for therapeutic and/or diagnostic purposes in humans.

[0326] Antibodies may be administered by injection systemically, such as by intravenous injection; or by injection or application to the relevant site, such as by direct injection into a tumor, or direct application to the site when the site is exposed in surgery; or by topical application, such as if the disorder is on the skin, for example.

[0327] For *in vivo* use, particularly for injection into humans, in some embodiments it is desirable to decrease the antigenicity of the antibody. An immune response of a recipient against the antibody may potentially decrease the period of time that the therapy is effective. Methods of humanizing antibodies are known in the art. The humanized antibody can be the product of an animal having transgenic human immunoglobulin genes, *e.g.*, constant region genes (*e.g.*, Grosveld and Kolias, 1992; Murphy and Carter, 1993; Pinkert, 1994; and International Patent Applications WO 90/10077 and WO 90/04036). Alternatively, the antibody of interest can be engineered by recombinant DNA techniques to substitute the CH1, CH2, CH3, hinge domains, and/or the framework domain with the corresponding human sequence (see, *e.g.*, WO 92/02190). Both polyclonal and monoclonal antibodies made in non-human animals may be "humanized" before administration to human subjects.

[0328] Chimeric immunoglobulin genes constructed with immunoglobulin cDNA are known in the art (Liu et al. 1987a; Liu et al. 1987b). Messenger RNA is isolated from a hybridoma or other cell producing the antibody and used to produce cDNA. The cDNA of interest can be amplified by the polymerase chain reaction using specific primers (U.S. Patent nos. 4,683,195 and 4,683,202). Alternatively, a library is made and screened to isolate the sequence of interest. The DNA sequence encoding the variable region of the antibody is then fused to human constant region sequences. The sequences of human constant regions genes are known in the art (Kabat et al., 1991). Human C region genes are readily available from known clones. The choice of isotype will be guided by the desired effector functions, such as complement fixation, or antibody-dependent cellular cytotoxicity. IgG1, IgG3 and IgG4 isotypes, and either of the kappa or lambda human light chain constant regions can be used. The chimeric, humanized antibody is then expressed by conventional methods.

[0329] Consensus sequences of heavy ("H") and light ("L") J regions can be used to design oligonucleotides for use as primers to introduce useful restriction sites into the J region for subsequent linkage of V region segments to human C region segments. C region

cDNA can be modified by site directed mutagenesis to place a restriction site at the analogous position in the human sequence.

[0330] A convenient expression vector for producing antibodies is one that encodes a functionally complete human CH or CL immunoglobulin sequence, with appropriate restriction sites engineered so that any VH or VL sequence can be easily inserted and expressed, such as plasmids, retroviruses, YACs, or EBV derived episomes, and the like. In such vectors, splicing usually occurs between the splice donor site in the inserted J region and the splice acceptor site preceding the human C region, and also at the splice regions that occur within the human CH exons. Polyadenylation and transcription termination occur at native chromosomal sites downstream of the coding regions. The resulting chimeric antibody can be joined to any strong promoter, including retroviral LTRs, *e.g.*, SV-40 early promoter, (Okayama, et al. 1983), Rous sarcoma virus LTR (Gorman et al. 1982), and Moloney murine leukemia virus LTR (Grosschedl et al. 1985), or native immunoglobulin promoters.

[0331] In yet other embodiments, the antibodies can be fully human antibodies. For example, xenogenic antibodies, which are produced in animals that are transgenic for human antibody genes, can be employed. By xenogenic human antibodies is meant antibodies that are fully human antibodies, with the exception that they are produced in a non-human host that has been genetically engineered to express human antibodies. (*e.g.*, WO 98/50433; WO 98,24893 and WO 99/53049).

[0332] Antibody fragments, such as Fv, F(ab')<sub>2</sub> and Fab can be prepared by cleavage of the intact protein, *e.g.*, by protease or chemical cleavage. These fragments can include heavy and light chain variable regions. Alternatively, a truncated gene can be designed, *e.g.*, a chimeric gene encoding a portion of the F(ab')<sub>2</sub> fragment that includes DNA sequences encoding the CH1 domain and hinge region of the H chain, followed by a translational stop codon. The antibodies of the present invention may be administered alone or in combination with other molecules for use as a therapeutic, for example, by linking the antibody to cytotoxic agent, as discussed above, or to a radioactive molecule. Radioactive antibodies that are specific to a cancer cell, disease cell, or virus-infected cell may be able to deliver a sufficient dose of radioactivity to kill such cancer cell, disease cell, or virus-infected cell. The antibodies of the present invention can also be used in assays for detection of the subject polypeptides. In some embodiments, the assay is a binding assay that detects binding of a polypeptide with an antibody specific for the polypeptide; the subject polypeptide or antibody can be immobilized, while the subject polypeptide and/or antibody can be detectably-labeled.



For example, the antibody can be directly labeled or detected with a labeled secondary antibody. That is, suitable, detectable labels for antibodies include direct labels, which label the antibody to the protein of interest, and indirect labels, which label an antibody that recognizes the antibody to the protein of interest.

[0333] These labels include radioisotopes, including, but not limited to  $^{64}\text{Cu}$ ,  $^{67}\text{Cu}$ ,  $^{90}\text{Y}$ ,  $^{124}\text{I}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{137}\text{Cs}$ ,  $^{186}\text{Re}$ ,  $^{211}\text{At}$ ,  $^{212}\text{Bi}$ ,  $^{213}\text{Bi}$ ,  $^{223}\text{Ra}$ ,  $^{241}\text{Am}$ , and  $^{244}\text{Cm}$ ; enzymes having detectable products (*e.g.*, luciferase,  $\beta$ -galactosidase, and the like); fluorescers and fluorescent labels, *e.g.*, as provided herein; fluorescence emitting metals, *e.g.*,  $^{152}\text{Eu}$ , or others of the lanthanide series, attached to the antibody through metal chelating groups such as EDTA; chemiluminescent compounds, *e.g.*, luminol, isoluminol, or acridinium salts; and bioluminescent compounds, *e.g.*, luciferin, or aequorin (green fluorescent protein), specific binding molecules, *e.g.*, magnetic particles, microspheres, nanospheres, and the like.

[0334] Alternatively, specific-binding pairs may be used, involving, *e.g.*, a second stage antibody or reagent that is detectably-labeled and that can amplify the signal. For example, a primary antibody can be conjugated to biotin, and horseradish peroxidase-conjugated streptavidin added as a second stage reagent. Digoxin and antidigoxin provide another such pair. In other embodiments, the secondary antibody can be conjugated to an enzyme such as peroxidase in combination with a substrate that undergoes a color change in the presence of the peroxidase. The absence or presence of antibody binding can be determined by various methods, including flow cytometry of dissociated cells, microscopy, radiography, or scintillation counting. Such reagents and their methods of use are well known in the art.

#### *e) Peptide Aptamers*

[0335] Another suitable agent for modulating an activity of a subject polypeptide is a peptide aptamer. Peptide aptamers are peptides or small polypeptides that act as dominant inhibitors of protein function. Peptide aptamers specifically bind to target proteins, blocking their functional ability (Kolonin and Finley, 1998). Due to the highly selective nature of peptide aptamers, they can be used not only to target a specific protein, but also to target specific functions of a given protein (*e.g.*, a signaling function). Further, peptide aptamers can be expressed in a controlled fashion by use of promoters which regulate expression in a temporal, spatial or inducible manner. Peptide aptamers act dominantly, therefore, they can be used to analyze proteins for which loss-of-function mutants are not available.

[0336] Peptide aptamers that bind with high affinity and specificity to a target protein can be isolated by a variety of techniques known in the art. Peptide aptamers can be isolated from random peptide libraries by yeast two-hybrid screens (Xu et al., 1997). They can also be isolated from phage libraries (Hoogenboom et al., 1998) or chemically generated peptides/libraries.

#### *Therapeutic Applications*

[0337] The instant invention provides various therapeutic methods. In some embodiments, methods of modulating, including increasing and inhibiting, a biological activity of a subject protein are provided. In some embodiments, methods of modulating an enzymatic activity of a subject protein are provided. In some embodiments, methods of increasing the level of enzymatically active subject protein are provided, while in some embodiments, methods of decreasing a level of enzymatically active subject protein are provided.

[0338] In some embodiments, methods of modulating enzymatic activity of a subject protein are provided. In other embodiments, methods of modulating a signal transduction activity of a subject protein are provided. In further embodiments, methods of modulating interaction of a subject protein with another, interacting protein or other macromolecule (*e.g.*, DNA, carbohydrate, lipid) are provided. In further embodiments, methods of modulating transport activity of a subject protein are provided. In further embodiments, methods of modulating phospholipase activity of a subject protein are provided. In further embodiments, methods of modulating polymerase activity of a subject protein are provided. In further embodiments, methods of modulating nuclease activity of a subject protein are provided.

[0339] As mentioned above, an effective amount of the active agent (*e.g.*, small molecule, antibody specific for a subject polypeptide, a subject polypeptide, or a subject polynucleotide) is administered to the host, where "effective amount" means a dosage sufficient to produce a desired effect or result. In some embodiments, the desired result is at least a reduction in a given biological activity of a subject polypeptide as compared to a control, for example, a decreased level of enzymatically active subject protein in the individual, or in a localized anatomical site in the individual. In further embodiments, the desired result is at least an increase in a biological activity of a subject polypeptide as compared to a control, for example an increased level of enzymatically active subject protein in the individual, or in a localized anatomical site in the individual.

[0340] Typically, the compositions of the instant invention will contain from less than about 1% to about 95% of the active ingredient, about 10% to about 50%. Generally, between about 100 mg and about 500 mg will be administered to a child and between about 500 mg and about 5 grams will be administered to an adult.

[0341] Other effective dosages can be readily determined by one of ordinary skill in the art through routine trials establishing dose response curves, for example, the amount of agent necessary to increase a level of active subject polypeptide can be calculated from *in vitro* experimentation. Those of skill will readily appreciate that dose levels can vary as a function of the specific compound, the severity of the symptoms, and the susceptibility of the subject to side effects, and preferred dosages for a given compound are readily determinable by those of skill in the art by a variety of means. For example, in order to calculate the polypeptide, polynucleotide, or modulator dose, those skilled in the art can use readily available information with respect to the amount necessary to have the desired effect, depending upon the particular agent used.

[0342] The active agent(s) can be administered to the host via any convenient means capable of resulting in the desired result. Administration is generally by injection and often by injection to a localized area. The frequency of administration will be determined by the care given based on patient responsiveness. For example, the agents may be administered daily, weekly, or as conventionally determined appropriate.

[0343] A variety of hosts are treatable according to the subject methods. The host, or patient, may be from any animal species, and will generally be mammalian, *e.g.*, primate sp., *e.g.*, monkeys, chimpanzees, and particularly humans; rodents, including mice, rats and hamsters, guinea pig; rabbits; cattle, including equines, bovines, pig, sheep, goat, canines; felines; etc. Animal models are of interest for experimental investigations, providing a model for treatment of human disease.

### *1. Proliferative Conditions*

[0344] In some embodiments, a protein of the present invention is involved in the control of cell proliferation, and an agent of the invention inhibits undesirable cell proliferation. Such agents are useful for treating disorders that involve abnormal cell proliferation, including, but not limited to, cancer, psoriasis, and scleroderma. Whether a particular agent and/or therapeutic regimen of the invention is effective in reducing unwanted cellular proliferation, *e.g.*, in the context of treating cancer, can be determined using standard methods. For example, the number of cancer cells in a biological sample (*e.g.*, blood, a

biopsy sample, and the like), can be determined. The tumor mass can be determined using standard radiological or biochemical methods.

[0345] Tumors that can be treated using the methods of the instant invention include carcinomas, *e.g.*, colorectal, prostate, breast, bone, kidney, skin, melanoma, ductal, endometrial, stomach or other organ of the gastrointestinal tract, pancreatic, mesothelioma, dysplastic oral mucosa, invasive oral cancer, tracheal cancer, non-small cell lung carcinoma ("NSCL"), transitional and squamous cell urinary carcinoma; brain cancer and neurological malignancies, *e.g.*, neuroblastoma, glioblastoma, astrocytoma, and gliomas; lymphomas and leukemias such as myeloid leukemia, myelogenous leukemia, hematological malignancies, such as childhood acute leukemia, non-Hodgkin's lymphomas, chronic lymphocytic leukemia, malignant cutaneous T-cell lymphoma, mycosis fungoides, non-MF cutaneous T-cell lymphoma, lymphomatoid papulosis, T-cell rich cutaneous lymphoid hyperplasia, bullous pemphigoid, discoid lupus erythematosus, lichen planus, and human follicular lymphoma; cancers of the reproductive system, *e.g.*, cervical and ovarian cancers and testicular cancers; liver cancers including hepatocellular carcinoma ("HCC") and tumors of the biliary duct; multiple myelomas; tumors of the esophageal tract; other lung cancers and tumors including small cell and clear cell; Hodgkin's lymphomas; adenocarcinoma; and sarcomas, including soft tissue sarcomas.

## 2. Immunotherapeutic Approaches to Proliferative Conditions

[0346] The polynucleotides, polypeptides, and modulators of the present invention find use in immunotherapy of hyperproliferative disorders, including cancer, neoplastic, and paraneoplastic disorders. That is, the subject molecules can correspond to tumor antigens, of which 1770 have been identified to date (Yu and Restifo, 2002). Immunotherapeutic approaches include passive immunotherapy and vaccine therapy and can accomplish both generic and antigen-specific cancer immunotherapy.

[0347] Passive immunity approaches involve antibodies of the invention that are directed toward specific tumor-associated antigens. Such antibodies can eradicate systemic tumors at multiple sites, without eradicating normal cells. In some embodiments, the antibodies are combined with radioactive components, as provided above, for example, combining the antibody's ability to specifically target tumors with the added lethality of the radioisotope to the tumor DNA.

[0348] Useful antibodies comprise a discrete epitope or a combination of nested epitopes, *i.e.*, a 10-mer epitope and associated peptide multimers incorporating all potential 8-

mers and 9-mers, or overlapping epitopes (Dutoit et al., 2002). Thus a single antibody can interact with one or more epitopes. Further, the antibody can be used alone or in combination with different antibodies, that all recognize either a single or multiple epitopes.

[0349] Neutralizing antibodies can provide therapy for cancer and proliferative disorders. Neutralizing antibodies that specifically recognize a secreted protein or peptide of the invention can bind to the secreted protein or peptide, *e.g.*, in a bodily fluid or the extracellular space, thereby modulating the biological activity of the secreted protein or peptide. For example, neutralizing antibodies specific for secreted proteins or peptides that play a role in stimulating the growth of cancer cells can be useful in modulating the growth of cancer cells. Similarly, neutralizing antibodies specific for secreted proteins or peptides that play a role in the differentiation of cancer cells can be useful in modulating the differentiation of cancer cells.

[0350] Vaccine therapy involves the use of polynucleotides, polypeptides, or agents of the invention as immunogens for tumor antigens (Machiels et al., 2002). For example, peptide-based vaccines of the invention include unmodified subject polypeptides, fragments thereof, and MHC class I and class II-restricted peptide (Knutson et al., 2001), comprising, for example, the disclosed sequences with universal, nonspecific MHC class II-restricted epitopes. Peptide-based vaccines comprising a tumor antigen can be given directly, either alone or in conjunction with other molecules. The vaccines can also be delivered orally by producing the antigens in transgenic plants that can be subsequently ingested (U.S. Patent No. 6,395,964).

[0351] In some embodiments, antibodies themselves can be used as antigens in anti-idiotypic vaccines. That is, administering an antibody to a tumor antigen stimulates B cells to make antibodies to that antibody, which in turn recognize the tumor cells

[0352] Nucleic acid-based vaccines can deliver tumor antigens as polynucleotide constructs encoding the antigen. Vaccines comprising genetic material, such as DNA or RNA, can be given directly, either alone or in conjunction with other molecules. Administration of a vaccine expressing a molecule of the invention, *e.g.*, as plasmid DNA, leads to persistent expression and release of the therapeutic immunogen over a period of time, helping to control unwanted tumor growth.

[0353] In some embodiments, nucleic acid-based vaccines encode subject antibodies. In such embodiments, the vaccines (*e.g.*, DNA vaccines) can include post-transcriptional regulatory elements, such as the post-transcriptional regulatory acting RNA element (WPRE)

derived from Woodchuck Hepatitis Virus. These post-transcriptional regulatory elements can be used to target the antibody, or a fusion protein comprising the antibody and a co-stimulatory molecule, to the tumor microenvironment (Pertl et al., 2003).

[0354] Besides stimulating anti-tumor immune responses by inducing humoral responses, vaccines of the invention can also induce cellular responses, including stimulating T-cells that recognize and kill tumor cells directly. For example, nucleotide-based vaccines of the invention encoding tumor antigens can be used to activate the CD8<sup>+</sup> cytotoxic T lymphocyte arm of the immune system.

[0355] In some embodiments, the vaccines activate T-cells directly, and in others they enlist antigen-presenting cells to activate T-cells. Killer T-cells are primed, in part, by interacting with antigen-presenting cells, *i.e.*, dendritic cells. In some embodiments, plasmids comprising the nucleic acid molecules of the invention enter antigen-presenting cells, which in turn display the encoded tumor-antigens that contribute to killer T-cell activation. Again, the tumor antigens can be delivered as plasmid DNA constructs, either alone or with other molecules.

[0356] In further embodiments, RNA can be used. For example, dendritic cells can be transfected with RNA encoding tumor antigens (Heiser et al., 2002; Mitchell and Nair, 2000). This approach overcomes the limitations of obtaining sufficient quantities of tumor material, extending therapy to patients otherwise excluded from clinical trials. For example, a subject RNA molecule isolated from tumors can be amplified using RT-PCR. In some embodiments, the RNA molecule of the invention is directly isolated from tumors and transfected into dendritic cells with no intervening cloning steps.

[0357] In some embodiments the molecules of the invention are altered such that the peptide antigens are more highly antigenic than in their native state. These embodiments address the need in the art to overcome the poor *in vivo* immunogenicity of most tumor antigens by enhancing tumor antigen immunogenicity via modification of epitope sequences (Yu and Restifo, 2002).

[0358] Another recognized problem of cancer vaccines is the presence of preexisting neutralizing antibodies. Some embodiments of the present invention overcome this problem by using viral vectors from non-mammalian natural hosts, *i.e.*, avian pox viruses. Alternative embodiments that also circumvent preexisting neutralizing antibodies include genetically engineered influenza viruses, and the use of "naked" plasmid DNA vaccines that contain DNA with no associated protein (Yu and Restifo, 2002).

[0359] All of the immunogenic methods of the invention can be used alone or in combination with other conventional or unconventional therapies. For example, immunogenic molecules can be combined with other molecules that have a variety of antiproliferative effects, or with additional substances that help stimulate the immune response, *i.e.*, adjuvants or cytokines.

[0360] For example, in some embodiments, nucleic acid vaccines encode an alphaviral replicase enzyme, in addition to tumor antigens. This recently discovered approach to vaccine therapy successfully combines therapeutic antigen production with the induction of the apoptotic death of the tumor cell (Yu and Restifo, 2002).

[0361] In certain other embodiments, a DNA or RNA vaccine of the present invention can also be directed against the production of blood vessels in the vicinity of the tumor, a process called antiangiogenesis, thereby depriving the cancer cells of nutrients. For example, the antiangiogenic molecules angiostatin (a fragment of plasminogen), endostatin (a fragment of collagen XVIII), interferon- $\gamma$ , interferon- $\gamma$  inducible protein 10, interleukin 12, thrombospondin, platelet factor-4, calreticulin, or its protein fragment vasostatin can be used to treat tumors by suppressing neovascularization and thereby inhibiting growth (Cheng et al., 2001). The antiangiogenesis approach can be used alone, or in conjunction with molecules directed to tumor antigens.

[0362] Furthermore, adjuvants can be used in conjunction with the antibodies and vaccines disclosed herein. Adjuvants help boost the general immune response, for example, concentrating immune cells to the specific area where they are needed. They can be added to a cancer vaccine itself or administered separately, and in some embodiments, a viral vector can be engineered to display adjuvant proteins on its surface.

[0363] Cytokines can also be used to help stimulate immune response. Cytokines act as chemical messengers, recruiting immune cells that help the killer T-cells to the site of attack. An example of a cytokine is granulocyte-macrophage colony-stimulating factor (GM-CSF), which stimulates the proliferation of antigen-presenting cells, thus boosting an organism's response to a cancer vaccine. As with adjuvants, cytokines can be used in conjunction with the antibodies and vaccines disclosed herein. For example, they can be incorporated into the antigen-encoding plasmid or introduced via a separate plasmid, and in some embodiments, a viral vector can be engineered to display cytokines on its surface.

### 3. *Inflammation and Immunity*

[0364] In other embodiments, *e.g.*, where the subject polypeptide is involved in modulating inflammation or immune function, the invention provides agents for treating such inflammation or immune disorders. Disease states that are treatable using formulations of the invention include various types of arthritis such as rheumatoid arthritis and osteoarthritis, autoimmune thyroiditis, various chronic inflammatory conditions of the skin, such as psoriasis, the intestine, such as inflammatory bowel disease (IBD), insulin-dependent diabetes, autoimmune diseases such as multiple sclerosis (MS), intestinal immune disorders and systemic lupus erythematosus (SLE), allergic diseases, transplant rejections, adult respiratory distress syndrome, atherosclerosis, ischemic diseases due to closure of the peripheral vasculature, cardiac vasculature, and vasculature in the central nervous system (CNS). After reading the present disclosure, those skilled in the art will recognize other disease states and/or symptoms which might be treated and/or mitigated by the administration of formulations of the present invention.

[0365] Neutralizing antibodies can provide immunosuppressive therapy for inflammatory and autoimmune disorders. Neutralizing antibodies can be used to treat disorders such as, for example, multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, transplant rejection, and psoriasis. Neutralizing antibodies that specifically recognize a secreted protein or peptide of the invention can bind to the secreted protein or peptide, *e.g.*, in a bodily fluid or the extracellular space, thereby modulating the biological activity of the secreted protein or peptide. For example, neutralizing antibodies specific for secreted proteins or peptides that play a role in activating immune cells are useful as immunosuppressants.

### 4. *Disorders Related to Cell Death*

[0366] Where a polypeptide of the invention is involved in modulating cell death, an agent of the invention is useful for treating conditions or disorders relating to cell death (*e.g.*, DNA damage, cell death, apoptosis). Cell death-related indications that can be treated using the methods of the invention to reduce cell death in a eukaryotic cell, include, but are not limited to, cell death associated with Alzheimer's disease, Parkinson's disease, rheumatoid arthritis, autoimmune thyroiditis, septic shock, sepsis, stroke, central nervous system inflammation, intestinal inflammation, osteoporosis, ischemia, reperfusion injury, cardiac muscle cell death associated with cardiovascular disease, polycystic kidney disease, cell death of endothelial cells in cardiovascular disease, degenerative liver disease, multiple



sclerosis, amyotrophic lateral sclerosis, cerebellar degeneration, ischemic injury, cerebral infarction, myocardial infarction, acquired immunodeficiency syndrome (AIDS), myelodysplastic syndromes, aplastic anemia, male pattern baldness, and head injury damage. Also included are conditions in which DNA damage to a cell is induced by external conditions, including but not limited to irradiation, radiomimetic drugs, hypoxic injury, chemical injury, and damage by free radicals. Also included are any hypoxic or anoxic conditions, *e.g.*, conditions relating to or resulting from ischemia, myocardial infarction, cerebral infarction, stroke, bypass heart surgery, organ transplantation, and neuronal damage, etc.

[0367] DNA damage can be detected using any known method, including, but not limited to, a Comet assay (commercially available from Trevigen, Inc.), which is based on alkaline lysis of labile DNA at sites of damage; and immunological assays using antibodies specific for aberrant DNA structures, *e.g.*, 8-OHdG.

[0368] Cell death can be measured using any known method, and is generally measured using any of a variety of known methods for measuring cell viability. Such assays are generally based on entry into the cell of a detectable compound (or a compound that becomes detectable upon interacting with, or being acted on by, an intracellular component) that would normally be excluded from a normal, living cell by its structurally and functionally intact cell membrane. Such compounds include substrates for intracellular enzymes, including, but not limited to, a fluorescent substrate for esterase; dyes that are excluded from living cells, including, but not limited to, trypan blue; and DNA-binding compounds, including, but not limited to, an ethidium compound such as ethidium bromide and ethidium homodimer, and propidium iodide.

[0369] Apoptosis, or programmed cell death, is a regulated process leading to cell death via a series of well-defined morphological changes. Programmed cell death provides a balance for cell growth and multiplication, eliminating unnecessary cells. The default state of the cell is to remain alive. A cell enters the apoptotic pathway when an essential factor is removed from the extracellular environment or when an internal signal is activated. Genes and proteins of the invention that suppress the growth of tumors by activating cell death provide the basis for treatment strategies for hyperproliferative disorders and conditions.

[0370] Apoptosis can be assayed using any known method. Assays can be conducted on cell populations or an individual cell, and include morphological assays and biochemical assays. A non-limiting example of a method of determining the level of apoptosis in a cell

population is TUNEL (TdT-mediated dUTP nick-end labeling) labeling of the 3'-OH free end of DNA fragments produced during apoptosis (Gavrieli et al., 1992). The TUNEL method consists of catalytically adding a nucleotide, which has been conjugated to a chromogen system, a fluorescent tag, or the 3'-OH end of the 180-bp (base pair) oligomer DNA fragments, in order to detect the fragments. The presence of a DNA ladder of 180-bp oligomers is indicative of apoptosis. Procedures to detect cell death based on the TUNEL method are available commercially, *e.g.*, from Boehringer Mannheim (Cell Death Kit) and Oncor (Apoptag Plus).

[0371] Another marker that is currently available is annexin, sold under the trademark APOPTTEST™. This marker is used in the "Apoptosis Detection Kit," which is also commercially available, *e.g.*, from R&D Systems. During apoptosis, a cell membrane's phospholipid asymmetry changes such that the phospholipids are exposed on the outer membrane. Annexins are a homologous group of proteins that bind phospholipids in the presence of calcium. A second reagent, propidium iodide (PI), is a DNA binding fluorochrome. When a cell population is exposed to both reagents, apoptotic cells stain positive for annexin and negative for PI, necrotic cells stain positive for both, live cells stain negative for both. Other methods of testing for apoptosis are known in the art and can be used, including, *e.g.*, the method disclosed in U.S. Patent No. 6,048,703.

#### 5. Other Pathological Conditions

[0372] Other pathological conditions that can be treated using the methods of the instant invention include disorders of hematopoiesis, cell differentiation, disorders of ion channels, *e.g.*, cystic fibrosis, and tissue or organ hypertrophy, viral disorders, including acquired immunodeficiency syndrome (AIDS), angiogenesis, metastasis, metabolic disorders such as diabetes and obesity, cardiovascular disorders such as congestive heart failure and stroke, male erectile dysfunction, and the disorders described throughout the specification.

#### Investigative Applications

[0373] The subject nucleic acid compositions find use in a variety of different investigative applications. Applications of interest include identifying genomic DNA sequence using molecules of the invention, identifying homologs of molecules of the invention, creating a source of novel promoter elements, identifying expression regulatory factors, creating a source of probes and primers for hybridization applications, identifying expression patterns in biological specimens; preparing cell or animal models to investigate

the function of the molecules of the invention, and preparing *in vitro* models to investigate the function of the molecules of the invention.

### **Genomic DNA Sequences**

[0374] Human genomic polynucleotide sequences corresponding to molecules of the present invention are identified by conventional means, such as, for example, by probing a genomic DNA library with all or a portion of the polynucleotide sequences.

[0375] Homologs are identified by any of a number of methods. By using probes, particularly labeled probes of DNA sequences, one can isolate homologous or related genes, as described in detail above. Briefly, a fragment of the provided cDNA can be used as a hybridization probe against a cDNA library from the target organism of interest, under various stringency conditions, *e.g.*, low stringency conditions. The probe can be a large fragment, or one or more short degenerate primers, and is typically labeled. Sequence identity can be determined by hybridization under stringent conditions, as described in detail above. Nucleic acids having a region of substantial identity or sequence similarity to the provided nucleic acid sequences, for example allelic variants, related genes, or genetically altered versions of the gene, bind to the provided sequences under less stringent hybridization conditions.

### **Promoter Elements and Expression Regulatory Factors**

[0376] The sequence of the 5' flanking region can be utilized as promoter elements, including enhancer binding sites that provide for tissue-specific expression and developmental regulation in tissues where the subject genes are expressed, providing promoters that mimic the native pattern of expression. Naturally occurring polymorphisms in the promoter region are useful for determining natural variations in expression, particularly those that may be associated with disease. Promoters or enhancers that regulate the transcription of the polynucleotides of the present invention are obtainable by use of PCR techniques using human tissues, and one or more of the present primers.

[0377] Alternatively, mutations can be introduced into the promoter region to determine the effect of altering expression in experimentally defined systems. Methods for the identification of specific DNA motifs involved in the binding of transcriptional factors are known in the art, for example sequence similarity to known binding motifs, and gel retardation studies (Blackwell et al., 1995; Mortlock et al., 1996; Joulin and Richard-Foy, 1995).

[0378] The regulatory sequences can be used to identify *cis* acting sequences required for transcriptional or translational regulation of expression, especially in different tissues or stages of development, and to identify *cis* acting sequences and *trans*-acting factors that regulate or mediate expression. Such transcription or translational control regions can be operably linked to a gene in order to promote expression of wild type genes or of proteins of interest in cultured cells, embryonic, fetal or adult tissues, and for gene therapy (Hooper, 1993).

#### **Primers and Probes**

[0379] Small DNA fragments are useful as primers for reactions that involve nucleic acid hybridization, as described in detail above. Briefly, pairs of primers will be used in amplification reactions, such as PCR. Amplification primers hybridize to complementary strands of DNA, for example, under stringent conditions, and will prime towards each other. In some embodiments a pair of primers will generate an amplification product of at least about 50 nt, or at least about 100 nt. Algorithms for the selection of primer sequences are generally known, and are available in commercial software packages.

[0380] The nucleotides can also be used as probes to identify genomic DNA or gene expression in a biological specimen, as described above and as is well established in the art. Briefly, DNA or mRNA is isolated from a cell sample. Detection of mRNA hybridizing to the subject sequence is indicative of gene expression in the sample. The mRNA can be amplified by RT-PCR, using reverse transcriptase to form a complementary DNA strand, followed by polymerase chain reaction amplification using primers specific for the subject DNA sequences. Alternatively, the mRNA sample is separated by gel electrophoresis, transferred to a suitable support, *e.g.*, nitrocellulose, nylon, *etc.*, and then probed with a fragment of the subject nucleotides as a probe. Other techniques, such as oligonucleotide ligation assays, *in situ* hybridizations, and hybridization to probes arrayed on a solid chip may also find use.

#### **Targeted Mutations for *In Vivo* and *In Vitro* Models**

[0381] The sequence of a gene according to the subject invention, including flanking promoter regions and coding regions, can be mutated in various ways known in the art to generate targeted changes, *i.e.*, changes in promoter strength, or sequence of the encoded protein, *etc.* The DNA sequence or protein product of such a mutation will usually be substantially similar to the sequences provided herein. The sequence changes can be

substitutions, insertions, deletions, or a combination thereof. Deletions can further include larger changes, such as deletions of a domain or exon.

[0382] Techniques for *in vitro* mutagenesis of cloned genes are known. Examples of protocols for site specific mutagenesis may be found in Gustin et al., 1993; Barany 1985; Colicelli et al., 1985; Prentki et al., 1984. Methods for site specific mutagenesis can be found in Sambrook et al., 1989 (pp. 15.3-15.108); Weiner et al., 1993; Sayers et al. 1992; Jones and Winistorfer; Barton et al., 1990; Marotti and Tomich 1989; and Zhu, 1989. Such mutated genes can be used to study structure-function relationships of the subject proteins, or to alter properties of the protein that affect its function or regulation. Other modifications of interest include epitope tagging, *e.g.*, with hemagglutinin (HA), FLAG, or *c-myc*. For studies of subcellular localization, fluorescent fusion proteins can be used.

[0383] The subject nucleic acids can be used to generate transgenic, non-human animals and/or site-specific gene modifications in cell lines; suitable methods are known in the art (Grosveld and Kollias, 1992; Hooper, 1993; Murphy and Carter, 1993; Pinkert, 1994). Thus, in some embodiments, the invention provides a non-human transgenic animal comprising, as a transgene integrated into the genome of the animal, a nucleic acid molecule comprising a sequence encoding a subject polypeptide in operable linkage with a promoter, such that the subject polypeptide-encoding nucleic acid molecule is expressed in a cell of the animal. Either a complete or partial sequence of a gene native to the host can be introduced. Alternatively, a complete or partial sequence of a gene exogenous to the host animal, *e.g.*, a human sequence of the subject invention, can be introduced. Transgenic animals can be made through homologous recombination, where the endogenous locus is altered. Thus, DNA constructs for homologous recombination will comprise at least a portion of the human gene or of a gene native to the species of the host animal, wherein the gene has the desired genetic modification(s), and includes regions of homology to the target locus. Methods for generating mammalian cells having targeted gene modifications through homologous recombination are known in the art (Keown et al., 1990).

[0384] Alternatively, a nucleic acid construct is randomly integrated into the genome. Vectors for stable integration include plasmids, retroviruses and other animal viruses, and YACs. DNA constructs for random integration need not include regions of homology to mediate recombination.

[0385] Conveniently, markers for positive and negative selection are included. A detectable marker, such as *lac Z* can be introduced into a locus at which up-regulation of expression will result in a detectable change in phenotype.

[0386] Transformed ES or embryonic cells can be used to produce transgenic animals. An embryonic stem (ES) cell line can be a source of embryonic stem cells, or they can be newly obtained from a host animal, *e.g.*, a mouse, rat, or guinea pig. The cells are grown on an appropriate fibroblast-feeder layer or in the presence of leukemia inhibiting factor (LIF). Following transformation, the cells are plated for growth onto a feeder layer in an appropriate medium. Cells containing the relevant construct can be detected by employing a selective medium and analyzing them for the occurrence of homologous recombination or integration of the construct. Positive colonies can be used for embryo manipulation and blastocyst injection. Blastocysts are obtained from 4 to 6 week old super-ovulated females. The ES cells are trypsinized, and the modified cells are injected into the blastocoel of the blastocyst. After injection, the blastocysts are returned to each uterine horn of pseudopregnant female animals that proceed to term. The resulting offspring are screened for the construct. By providing for a different phenotype of the blastocyst and the genetically modified cells, chimeric progeny can be readily detected.

[0387] The chimeric animals are screened for the presence of the modified gene and males and females having the modification are mated to produce homozygous progeny. If the gene alterations cause lethality at some point in development, tissues or organs can be maintained as allogeneic or congenic grafts or transplants, or in *in vitro* culture. The transgenic animals can be any non-human mammal.

[0388] The modified cells or animals are useful in the study of gene function and regulation. For example, a series of small deletions and/or substitutions can be made in the host's native gene to determine the role of different exons in biological processes such as oncogenesis or signal transduction. Of interest is the use of genes to construct transgenic animal models for cancer, where expression of the subject protein is specifically reduced or absent. Specific constructs of interest include anti-sense constructs, which will block expression, expression of dominant negative mutations, and gene over-expression.

[0389] One can also provide for expression of the gene, *e.g.*, a subject gene, or variants thereof, in cells or tissues where it is not normally expressed, at levels not normally present in such cells or tissues, or at abnormal times of development. One can also generate host cells (including host cells in transgenic animals) that comprise a heterologous nucleic

acid molecule which encodes a polypeptide which functions to modulate expression of an endogenous promoter or other transcriptional regulatory region, or the biological activity of a subject polypeptide. The transgenic animals can also be used in functional studies, for example drug screening, to determine the effect of a candidate drug on a biological activity of

### **Examples**

[0390] The examples, which are intended to be purely exemplary of the invention and should therefore not be considered to limit the invention in any way, also describe and detail aspects and embodiments of the invention discussed above. The examples are not intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (*e.g.*, amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

[0391] While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications can be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

[0392] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed. Moreover, it must be understood that the invention is not limited to the particular embodiments described, as such may, of course, vary. Further, the terminology used to describe particular embodiments is not intended to be limiting, since the scope of the present invention will be limited only by its claims.

[0393] With respect to ranges of values, the invention encompasses each intervening value between the upper and lower limits of the range to at least a tenth of the lower limit's unit, unless the context clearly indicates otherwise. Further, the invention encompasses any other stated intervening values. Moreover, the invention also encompasses ranges excluding either or both of the upper and lower limits of the range, unless specifically excluded from the stated range.

[0394] Unless defined otherwise, the meanings of all technical and scientific terms used herein are those commonly understood by one of ordinary skill in the art to which this invention belongs. One of ordinary skill in the art will also appreciate that any methods and materials similar or equivalent to those described herein can also be used to practice or test the invention. Further, all publications mentioned herein are incorporated by reference.

[0395] It must be noted that, as used herein and in the appended claims, the singular forms "a," "or," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a subject polypeptide" includes a plurality of such polypeptides and reference to "the agent" includes reference to one or more agents and equivalents thereof known to those skilled in the art, and so forth.

[0396] Further, all numbers expressing quantities of ingredients, reaction conditions, % purity, polypeptide and polynucleotide lengths, and so forth, used in the specification and claims, are modified by the term "about," unless otherwise indicated. Accordingly, the numerical parameters set forth in the specification and claims are approximations that may vary depending upon the desired properties of the present invention. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits, applying ordinary rounding techniques. Nonetheless, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors from the standard deviation of its experimental measurement.

[0397] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

#### **Example 1 Expression in *E. coli***

[0398] Sequences can be expressed in *E. coli*. Any one or more of the sequences according to SEQ. ID. NOS. 1-187 and 375-484 can be expressed in *E. coli* by subcloning the entire coding region, or a selected portion thereof, into a prokaryotic expression vector. For example, the expression vector pQE16 from the QIA expression prokaryotic protein expression system (Qiagen, Valencia, CA) can be used. The features of this vector that make it useful for protein expression include an efficient promoter (phage T5) to drive



transcription, expression control provided by the lac operator system, which can be induced by addition of IPTG (isopropyl-beta-D-thiogalactopyranoside), and an encoded 6XHis tag coding sequence. The latter is a stretch of six histidine amino acid residues which can bind very tightly to a nickel atom. This vector can be used to express a recombinant protein with a 6XHis. tag fused to its carboxyl terminus, allowing rapid and efficient purification using Ni-coupled affinity columns.

[0399] The entire or the selected partial coding region can be amplified by PCR, then ligated into digested pQE16 vector. The ligation product can be transformed by electroporation into electrocompetent *E. coli* cells (for example, strain M15[pREP4] from Qiagen), and the transformed cells may be plated on ampicillin-containing plates. Colonies may then be screened for the correct insert in the proper orientation using a PCR reaction employing a gene-specific primer and a vector-specific primer. Also, positive clones can be sequenced to ensure correct orientation and sequence. To express the proteins, a colony containing a correct recombinant clone can be inoculated into L-Broth containing 100 µg/ml of ampicillin, and 25 µg/ml of kanamycin, and the culture allowed to grow overnight at 37 degrees C. The saturated culture may then be diluted 20-fold in the same medium and allowed to grow to an optical density of 0.5 at 600 nm. At this point, IPTG can be added to a final concentration of 1 mM to induce protein expression. After growing the culture for an additional 5 hours, the cells may be harvested by centrifugation at 3000 times *g* for 15 minutes.

[0400] The resultant pellet can be lysed with a mild, nonionic detergent in 20 mM Tris HCl (pH 7.5) (B PER.TM. Reagent from Pierce, Rockford, IL), or by sonication until the turbid cell suspension turns translucent. The resulting lysate can be further purified using a nickel-containing column (Ni-NTA spin column from Qiagen) under non-denaturing conditions. Briefly, the lysate will be adjusted to 300 mM NaCl and 10 mM imidazole, then centrifuged at 700 times *g* through the nickel spin column to allow the His-tagged recombinant protein to bind to the column. The column will be washed twice with wash buffer (for example, 50 mM NaH<sub>2</sub> PO<sub>4</sub>, pH 8.0; 300 mM NaCl; 20 mM imidazole) and eluted with elution buffer (for example, 50 mM NaH<sub>2</sub> PO<sub>4</sub>, pH 8.0; 300 mM NaCl; 250 mM imidazole). All the above procedures will be performed at 4 degrees C. The presence of a purified protein of the predicted size can be confirmed with SDS-PAGE.

### **Example 2: Expression in Mammalian Cells**

[0401] The sequences encoding the proteins of Example 1 can be cloned into the pENTR vector (Invitrogen) by PCR and transferred to the mammalian expression vector pDEST12.2 per manufacturer's instructions (Invitrogen). Introduction of the recombinant construct into the host cell can be effected by transfection with Fugene 6 (Roche) per manufacturer's instructions. The host cells containing one of polynucleotides of the invention can be used in conventional manners to produce the gene product encoded by the isolated fragment (in the case of an ORF). A number of types of cells can act as suitable host cells for expression of the proteins. Mammalian host cells include, for example, monkey COS cells, Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3 cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from *in vitro* culture of primary tissue, primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HaK or Jurkat cells.

### **Example 3: Expression in Cell-Free Translation Systems**

[0402] Cell-free translation systems can also be employed to produce proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors containing SP6 or T7 promoters for use with prokaryotic and eukaryotic hosts have been described (Sambrook et al., 1989). These DNA constructs can be used to produce proteins in a rabbit reticulocyte lysate system or in a wheat germ extract system.

[0403] Specific expression systems of interest include plant, bacterial, yeast, insect cell and mammalian cell derived expression systems. Expression systems in plants include those described in U.S. Patent No. 6,096,546 and U.S. Patent No. 6,127,145. Expression systems in bacteria include those described by Chang et al., 1978, Goeddel et al., 1979, Goeddel et al., 1980, EP 0 036,776, U.S. Patent No. 4,551,433; DeBoer et al., 1983, and Siebenlist et al., 1980.

[0404] Mammalian expression is further accomplished as described in Dijkema et al. 1985, Gorman et al., 1982, Boshart et al., 1985, and U.S. Patent No. 4,399,216. Other features of mammalian expression are facilitated as described in Ham and Wallace, Meth. Enz., 1979, Barnes and Sato, 1980, U.S. Patent Nos. 4,767,704, 4,657,866, 4,927,762, 4,560,655, WO 90/103430, WO 87/00195, and U.S. RE 30,985.

### **Example 4: Expression**

[0405] Primers can be designed to amplify the secreted factors using PCR and cloned into pENTR/D-TOPO vectors (Invitrogen, Carlsbad, CA). The secreted factors in pENTR/D-

TOPO can be cloned into the yeast expression vector pYES-DEST52 by Gateway LR reaction (Invitrogen, Carlsbad, CA). The resulting yeast expression vectors can be transformed into INVSc1 strain from Invitrogen to express the secreted factors according to the manufacturer's protocol (Invitrogen, Carlsbad CA). The expressed secreted factors will have a 6XHis tag at the C-terminal. Expressed protein can be purified with ProBond™ resin (Invitrogen, Carlsbad, CA).

[0406] Expression systems in yeast include those described in Hinnen et al., 1978, Ito et al., 1983, Kurtz et al., 1986, Kunze et al., 1985, Gleeson et al., 1986, Roggenkamp et al., 1986, Das et al., 1984, De Louvencourt et al., 1983, Van den Berg et al., 1990, Kunze et al., 1985, Cregg et al. 1985, U.S. Patent No. 4,837,148, U.S. Patent No. 4,929,555, Beach and Nurse, 1981, Davidow et al., 1985, Gaillardin et al., 1985, Ballance et al., 1983, Tilburn et al., 1983, Yelton et al., 1984, Kelly and Hynes, 1985, EP 0 244,234, and WO 91/00357.

**Example 5: Expression in a Baculovirus Expression System.**

[0407] The secreted factors in pENTR/D-TOPO can be cloned into Baculovirus expression vector pDEST10 by Gateway LR reaction (Invitrogen, Carlsbad, CA). The secreted factors can be expressed by the Bac-to-Bac expression system from Invitrogen (Carlsbad CA), briefly described as follows. The expression vectors containing the secreted factors are transformed into competent DH10Bac™ *E. coli* strain and selected for transposition. The resulting *E. coli* contain recombinant bacmid that contains the secreted factor. High molecular weight DNA can be isolated from the *E. coli* containing the recombinant bacmid and then transfected into insect cells with Cellfectin reagent. The expressed secreted factors will have a 6XHis tag at N-terminal. Expressed protein will be purified by ProBond™ resin (Invitrogen, Carlsbad, CA).

[0408] Expression of heterologous genes in insects can be accomplished as described in U.S. Patent No. 4,745,051; Doerfler et al., 1987; Friesen *et al.*, 1986; EP 0 127,839, EP 0 155,476, Vlak *et al.*, 1988, Miller *et al.*, 1988, Carbonell *et al.*, 1988, Maeda *et al.*, 1985, Lebacq-Verheyden *et al.*, 1988, Smith *et al.*, 1985, Miyajima *et al.*; and Martin *et al.*, 1988. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts have been previously described (Setlow et al., 1986, Luckow *et al.*, 1988; Miller *et al.*, 1986; Maeda *et al.*, 1985).

**Example 6: Primer Design**

[0409] To design the forward primer for PCR amplification, the melting point of the first 20 to 24 bases of the primer can be calculated by counting total A and T residues, then

multiplying by 2. To design the reverse primer for PCR amplification, the melting point of the first 20 to 24 bases of the reverse complement, with the sequences written from 5-prime to 3-prime can be calculated by counting the total G and C residues, then multiplying by 4. Both start and stop codons can be present in the final amplified clone. The length of the primers is such to obtain melting temperatures within 63 degrees C to 68 degrees C. Adding the bases "CACC" to the forward primer renders it compatible for cloning the PCR product with the TOPO pENTR/D (Invitrogen, CA).

#### **Example 7: Reverse Transcriptase Reaction**

[0410] cDNA can be prepared by the following method. Between 200 ng and 1.0 µg mRNA is added to 2 µl DMSO and the volume adjusted to 11 µl with DEPC-treated water. One µl Oligo dT is added to the tube, and the mixture is heated at 70° C for 5 min., quickly chilled on ice for 2 min., and the mixture is collected at the bottom of the tube by brief centrifugation. The following 1<sup>st</sup> strand components are then added to the mRNA mixture: 2 µl 10X Stratascript (Stratagene, CA) 1<sup>st</sup> strand buffer, 1 µl 0.1 M DTT, 1 µl 10 mM dNTP mix (10 mM each of dG, dA, dT and dCTP), 1 µl RNase inhibitor, 3 µl Stratascript RT (50 U/ µl). The contents are gently mixed and the mixture collected by brief centrifugation. The mixture is incubated in a 42° C water bath for 1 hour, placed in a 70° C water bath for 15 min. to stop the reaction, transferred to ice for 2 min., and centrifuged briefly in a microfuge to collect the reaction product at the bottom of the reaction vessel. Two µl RNase H is then added to the tube, the contents are mixed well, incubated at 37° C in a water bath for 20 min., and centrifuged briefly in a microfuge to collect the reaction product at the bottom of the reaction vessel. The reaction mixture can proceed directly to PCR or be stored at – 20° C.

#### **Example 8: Full Length PCR**

[0411] Full length PCR can be achieved by placing the products of the reaction described in Example 7, with primers diluted to 5µM in water, into a reaction vessel and adding a reaction mixture composed of 1x Taq buffer, 25 mM dNTP, 10 ng cDNA pool, TaqPlus (Stratagene, CA) (5u/ul), PfuTurbo (Stratagene, CA) (2.5u/ul), water. The contents of the reaction vessel are then mixed gently by inversion 5-6 times, placed into a reservoir where 2µl F<sub>1</sub>/R<sub>1</sub> primers are added, the plate sealed and placed in the thermocycler. The PCR reaction is comprised of the following eight steps. Step 1: 95° C for 3 min. Step 2: 94° C for 45 sec. Step 3: 0.5° C/sec to 56-60° C. Step 4: 56-60° C for 50 sec. Step 5: 72° C for 5 min. Step 6: Go to step 2, perform 35-40 cycles. Step 7: 72° C for 20 min. Step 8: 4° C.

[0412] The products can then be separated on a standard 0.8 to 1.0% agarose gel at 40 to 80 V, the bands of interest excised by cutting from the gel, and stored at -20° C until extraction. The material in the bands of interest can be purified with QIAquick 96 PCR Purification Kit (Qiagen, CA) according to the manufacturer instructions. Cloning can be performed with the Topo Vector pENTR/D-TOPO vector (Invitrogen, CA) according to the manufacturer's instructions.

## References

[0413] The specification is most thoroughly understood in light of the following references, all of which are hereby incorporated by reference in their entireties. The disclosures of the patents and other references cited above are also hereby incorporated by reference.

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**CLAIM**

1. A first nucleic acid molecule comprising a polynucleotide sequence chosen from at least one polynucleotide sequence according to SEQ. ID. NOS. 1- 187; SEQ. ID. NOS. 375-484, or a complement thereof, or from at least one polynucleotide sequence that encodes SEQ. ID. NOS. 188-374.

**ABSTRACT**

The invention provides novel polynucleotides, related polypeptides related nucleic acid and polypeptide compositions corresponding to novel human cDNA clones, and related modulators, such as antibodies and small molecule modulators. The invention also provides methods to make and use these polynucleotides, polypeptides, related compositions, and modulators. These methods include diagnostic, prophylactic and therapeutic applications. The compositions and methods of the invention are useful in treating proliferative disorders, *e.g.*, cancers, and inflammatory, immune, bacterial, and viral disorders.

**Table 1. Identification Numbers**

<b>FP ID</b>	<b>SEQ.ID.NO. (N1)</b>	<b>SEQ.ID.NO. (P1)</b>	<b>SEQ.ID.NO. (N0)</b>	<b>Clone ID</b>
HG1014903	SEQ.ID.NO. 1	SEQ.ID.NO. 188	SEQ.ID.NO. 375	PLT00014330A02.contig.a
HG1014904	SEQ.ID.NO. 2	SEQ.ID.NO. 189		PLT00014330A02.contig.b
HG1014905	SEQ.ID.NO. 3	SEQ.ID.NO. 190	SEQ.ID.NO. 376	PLT00014330A08.contig.a
HG1014906	SEQ.ID.NO. 4	SEQ.ID.NO. 191		PLT00014330A08.contig.b
HG1014907	SEQ.ID.NO. 5	SEQ.ID.NO. 192	SEQ.ID.NO. 377	PLT00014330A17.contig.a
HG1014908	SEQ.ID.NO. 6	SEQ.ID.NO. 193	SEQ.ID.NO. 378	PLT00014330A20.contig.a
HG1014909	SEQ.ID.NO. 7	SEQ.ID.NO. 194	SEQ.ID.NO. 379	PLT00014330B02.contig.a
HG1014910	SEQ.ID.NO. 8	SEQ.ID.NO. 195		PLT00014330B02.contig.b
HG1014911	SEQ.ID.NO. 9	SEQ.ID.NO. 196	SEQ.ID.NO. 380	PLT00014330B04.contig.a
HG1014912	SEQ.ID.NO. 10	SEQ.ID.NO. 197		PLT00014330B04.contig.b
HG1014913	SEQ.ID.NO. 11	SEQ.ID.NO. 198	SEQ.ID.NO. 381	PLT00014330B05.contig.a
HG1014914	SEQ.ID.NO. 12	SEQ.ID.NO. 199	SEQ.ID.NO. 382	PLT00014330B11.contig.a
HG1014915	SEQ.ID.NO. 13	SEQ.ID.NO. 200	SEQ.ID.NO. 383	PLT00014330B13.contig.a
HG1014916	SEQ.ID.NO. 14	SEQ.ID.NO. 201		PLT00014330B13.contig.b
HG1014917	SEQ.ID.NO. 15	SEQ.ID.NO. 202	SEQ.ID.NO. 384	PLT00014330B18.contig.a
HG1014918	SEQ.ID.NO. 16	SEQ.ID.NO. 203		PLT00014330B18.contig.b
HG1014919	SEQ.ID.NO. 17	SEQ.ID.NO. 204	SEQ.ID.NO. 385	PLT00014330C06.contig.a
HG1014920	SEQ.ID.NO. 18	SEQ.ID.NO. 205		PLT00014330C06.contig.b
HG1014921	SEQ.ID.NO. 19	SEQ.ID.NO. 206	SEQ.ID.NO. 386	PLT00014330C12.contig.a
HG1014922	SEQ.ID.NO. 20	SEQ.ID.NO. 207	SEQ.ID.NO. 387	PLT00014330C14.contig.a
HG1014923	SEQ.ID.NO. 21	SEQ.ID.NO. 208	SEQ.ID.NO. 388	PLT00014330C18.contig.a
HG1014924	SEQ.ID.NO. 22	SEQ.ID.NO. 209		PLT00014330C18.contig.b
HG1014925	SEQ.ID.NO. 23	SEQ.ID.NO. 210	SEQ.ID.NO. 389	PLT00014330D03.contig.a
HG1014926	SEQ.ID.NO. 24	SEQ.ID.NO. 211		PLT00014330D03.contig.b
HG1014927	SEQ.ID.NO. 25	SEQ.ID.NO. 212	SEQ.ID.NO. 390	PLT00014330D05.contig.a
HG1014928	SEQ.ID.NO. 26	SEQ.ID.NO. 213		PLT00014330D05.contig.b
HG1014929	SEQ.ID.NO. 27	SEQ.ID.NO. 214	SEQ.ID.NO. 391	PLT00014330D07.contig.a
HG1014930	SEQ.ID.NO. 28	SEQ.ID.NO. 215	SEQ.ID.NO. 392	PLT00014330D10.contig.a
HG1014931	SEQ.ID.NO. 29	SEQ.ID.NO. 216		PLT00014330D10.contig.b
HG1014932	SEQ.ID.NO. 30	SEQ.ID.NO. 217	SEQ.ID.NO. 393	PLT00014330D12.contig.a
HG1014933	SEQ.ID.NO. 31	SEQ.ID.NO. 218		PLT00014330D12.contig.b
HG1014934	SEQ.ID.NO. 32	SEQ.ID.NO. 219	SEQ.ID.NO. 394	PLT00014330D13.contig.a
HG1014935	SEQ.ID.NO. 33	SEQ.ID.NO. 220	SEQ.ID.NO. 395	PLT00014330D15.contig.a
HG1014936	SEQ.ID.NO. 34	SEQ.ID.NO. 221		PLT00014330D15.contig.b
HG1014937	SEQ.ID.NO. 35	SEQ.ID.NO. 222	SEQ.ID.NO. 396	PLT00014330D17.contig.a
HG1014938	SEQ.ID.NO. 36	SEQ.ID.NO. 223	SEQ.ID.NO. 397	PLT00014330E04.contig.a
HG1014939	SEQ.ID.NO. 37	SEQ.ID.NO. 224	SEQ.ID.NO. 398	PLT00014330E14.contig.a
HG1014940	SEQ.ID.NO. 38	SEQ.ID.NO. 225		PLT00014330E14.contig.b
HG1014941	SEQ.ID.NO. 39	SEQ.ID.NO. 226	SEQ.ID.NO. 399	PLT00014330E24.contig.a
HG1014942	SEQ.ID.NO. 40	SEQ.ID.NO. 227		PLT00014330E24.contig.b
HG1014943	SEQ.ID.NO. 41	SEQ.ID.NO. 228	SEQ.ID.NO. 400	PLT00014330F01.contig.a
HG1014944	SEQ.ID.NO. 42	SEQ.ID.NO. 229	SEQ.ID.NO. 401	PLT00014330F03.contig.a
HG1014945	SEQ.ID.NO. 43	SEQ.ID.NO. 230		PLT00014330F03.contig.b
HG1014946	SEQ.ID.NO. 44	SEQ.ID.NO. 231	SEQ.ID.NO. 402	PLT00014330F04.contig.a
HG1014947	SEQ.ID.NO. 45	SEQ.ID.NO. 232		PLT00014330F04.contig.b
HG1014948	SEQ.ID.NO. 46	SEQ.ID.NO. 233	SEQ.ID.NO. 403	PLT00014330F05.contig.a
HG1014949	SEQ.ID.NO. 47	SEQ.ID.NO. 234	SEQ.ID.NO. 404	PLT00014330F13.contig.a
HG1014950	SEQ.ID.NO. 48	SEQ.ID.NO. 235	SEQ.ID.NO. 405	PLT00014330G21.contig.a
HG1014951	SEQ.ID.NO. 49	SEQ.ID.NO. 236		PLT00014330G21.contig.b

FP ID	SEQ.ID.NO. (N1)	SEQ.ID.NO. (P1)	SEQ.ID.NO. (N0)	Clone ID
HG1014952	SEQ.ID.NO. 50	SEQ.ID.NO. 237		PLT00014330H05.contig.b
HG1014953	SEQ.ID.NO. 51	SEQ.ID.NO. 238	SEQ.ID.NO. 406	PLT00014330H06.contig.a
HG1014954	SEQ.ID.NO. 52	SEQ.ID.NO. 239	SEQ.ID.NO. 407	PLT00014330H12.contig.a
HG1014955	SEQ.ID.NO. 53	SEQ.ID.NO. 240		PLT00014330H12.contig.b
HG1014956	SEQ.ID.NO. 54	SEQ.ID.NO. 241	SEQ.ID.NO. 408	PLT00014330H14.contig.a
HG1014957	SEQ.ID.NO. 55	SEQ.ID.NO. 242		PLT00014330H14.contig.b
HG1014958	SEQ.ID.NO. 56	SEQ.ID.NO. 243	SEQ.ID.NO. 409	PLT00014330H18.contig.a
HG1014959	SEQ.ID.NO. 57	SEQ.ID.NO. 244		PLT00014330H18.contig.b
HG1014960	SEQ.ID.NO. 58	SEQ.ID.NO. 245	SEQ.ID.NO. 410	PLT00014330I11.contig.a
HG1014961	SEQ.ID.NO. 59	SEQ.ID.NO. 246	SEQ.ID.NO. 411	PLT00014330I12.contig.a
HG1014962	SEQ.ID.NO. 60	SEQ.ID.NO. 247		PLT00014330I12.contig.b
HG1014963	SEQ.ID.NO. 61	SEQ.ID.NO. 248	SEQ.ID.NO. 412	PLT00014330I13.contig.a
HG1014964	SEQ.ID.NO. 62	SEQ.ID.NO. 249		PLT00014330I13.contig.b
HG1014965	SEQ.ID.NO. 63	SEQ.ID.NO. 250	SEQ.ID.NO. 413	PLT00014330J10.contig.a
HG1014966	SEQ.ID.NO. 64	SEQ.ID.NO. 251		PLT00014330J10.contig.b
HG1014967	SEQ.ID.NO. 65	SEQ.ID.NO. 252	SEQ.ID.NO. 414	PLT00014330J14.contig.a
HG1014968	SEQ.ID.NO. 66	SEQ.ID.NO. 253		PLT00014330J14.contig.b
HG1014969	SEQ.ID.NO. 67	SEQ.ID.NO. 254	SEQ.ID.NO. 415	PLT00014330J15.contig.a
HG1014970	SEQ.ID.NO. 68	SEQ.ID.NO. 255	SEQ.ID.NO. 416	PLT00014330J21.contig.a
HG1014971	SEQ.ID.NO. 69	SEQ.ID.NO. 256		PLT00014330J21.contig.b
HG1014972	SEQ.ID.NO. 70	SEQ.ID.NO. 257	SEQ.ID.NO. 417	PLT00014330K01.contig.a
HG1014973	SEQ.ID.NO. 71	SEQ.ID.NO. 258	SEQ.ID.NO. 418	PLT00014330K08.contig.a
HG1014974	SEQ.ID.NO. 72	SEQ.ID.NO. 259		PLT00014330K08.contig.b
HG1014975	SEQ.ID.NO. 73	SEQ.ID.NO. 260	SEQ.ID.NO. 419	PLT00014330K09.contig.a
HG1014976	SEQ.ID.NO. 74	SEQ.ID.NO. 261		PLT00014330K09.contig.b
HG1014977	SEQ.ID.NO. 75	SEQ.ID.NO. 262	SEQ.ID.NO. 420	PLT00014330K15.contig.a
HG1014978	SEQ.ID.NO. 76	SEQ.ID.NO. 263		PLT00014330K15.contig.b
HG1014979	SEQ.ID.NO. 77	SEQ.ID.NO. 264	SEQ.ID.NO. 421	PLT00014330K24.contig.a
HG1014980	SEQ.ID.NO. 78	SEQ.ID.NO. 265	SEQ.ID.NO. 422	PLT00014330L01.contig.a
HG1015004	SEQ.ID.NO. 79	SEQ.ID.NO. 266	SEQ.ID.NO. 423	PLT00014330L24.contig.a
HG1014981	SEQ.ID.NO. 80	SEQ.ID.NO. 267	SEQ.ID.NO. 424	PLT00014330M02.contig.a
HG1014982	SEQ.ID.NO. 81	SEQ.ID.NO. 268		PLT00014330M02.contig.b
HG1014983	SEQ.ID.NO. 82	SEQ.ID.NO. 269	SEQ.ID.NO. 425	PLT00014330M08.contig.a
HG1014984	SEQ.ID.NO. 83	SEQ.ID.NO. 270		PLT00014330M08.contig.b
HG1014985	SEQ.ID.NO. 84	SEQ.ID.NO. 271	SEQ.ID.NO. 426	PLT00014330M15.contig.a
HG1014986	SEQ.ID.NO. 85	SEQ.ID.NO. 272	SEQ.ID.NO. 427	PLT00014330M17.contig.a
HG1014987	SEQ.ID.NO. 86	SEQ.ID.NO. 273		PLT00014330M17.contig.b
HG1014988	SEQ.ID.NO. 87	SEQ.ID.NO. 274	SEQ.ID.NO. 428	PLT00014330N10.contig.a
HG1014989	SEQ.ID.NO. 88	SEQ.ID.NO. 275		PLT00014330N10.contig.b
HG1014990	SEQ.ID.NO. 89	SEQ.ID.NO. 276	SEQ.ID.NO. 429	PLT00014330N12.contig.a
HG1014991	SEQ.ID.NO. 90	SEQ.ID.NO. 277		PLT00014330N12.contig.b
HG1014992	SEQ.ID.NO. 91	SEQ.ID.NO. 278	SEQ.ID.NO. 430	PLT00014330N13.contig.a
HG1014993	SEQ.ID.NO. 92	SEQ.ID.NO. 279		PLT00014330N13.contig.b
HG1014994	SEQ.ID.NO. 93	SEQ.ID.NO. 280	SEQ.ID.NO. 431	PLT00014330N22.contig.a
HG1014995	SEQ.ID.NO. 94	SEQ.ID.NO. 281		PLT00014330N22.contig.b
HG1014996	SEQ.ID.NO. 95	SEQ.ID.NO. 282	SEQ.ID.NO. 432	PLT00014330O03.contig.a
HG1014997	SEQ.ID.NO. 96	SEQ.ID.NO. 283	SEQ.ID.NO. 433	PLT00014330O07.contig.a
HG1014998	SEQ.ID.NO. 97	SEQ.ID.NO. 284		PLT00014330O07.contig.b
HG1015005	SEQ.ID.NO. 98	SEQ.ID.NO. 285	SEQ.ID.NO. 434	PLT00014330O18.contig.a
HG1015006	SEQ.ID.NO. 99	SEQ.ID.NO. 286		PLT00014330O18.contig.b
HG1014999	SEQ.ID.NO. 100	SEQ.ID.NO. 287	SEQ.ID.NO. 435	PLT00014330P07.contig.a
HG1015000	SEQ.ID.NO. 101	SEQ.ID.NO. 288		PLT00014330P07.contig.b

FP ID	SEQ.ID.NO. (N1)	SEQ.ID.NO. (P1)	SEQ.ID.NO. (N0)	Clone ID
HG1015001	SEQ.ID.NO. 102	SEQ.ID.NO. 289	SEQ.ID.NO. 436	PLT00014330P09.contig.a
HG1015002	SEQ.ID.NO. 103	SEQ.ID.NO. 290		PLT00014330P09.contig.b
HG1015003	SEQ.ID.NO. 104	SEQ.ID.NO. 291	SEQ.ID.NO. 437	PLT00014330P15.contig.a
HG1015007	SEQ.ID.NO. 105	SEQ.ID.NO. 292	SEQ.ID.NO. 438	PLT00014333A03.contig.a
HG1015008	SEQ.ID.NO. 106	SEQ.ID.NO. 293		PLT00014333A03.contig.b
HG1015009	SEQ.ID.NO. 107	SEQ.ID.NO. 294	SEQ.ID.NO. 439	PLT00014333A06.contig.a
HG1015010	SEQ.ID.NO. 108	SEQ.ID.NO. 295		PLT00014333A06.contig.b
HG1015011	SEQ.ID.NO. 109	SEQ.ID.NO. 296	SEQ.ID.NO. 440	PLT00014333A08.contig.a
HG1015012	SEQ.ID.NO. 110	SEQ.ID.NO. 297	SEQ.ID.NO. 441	PLT00014333A15.contig.a
HG1015013	SEQ.ID.NO. 111	SEQ.ID.NO. 298		PLT00014333A15.contig.b
HG1015014	SEQ.ID.NO. 112	SEQ.ID.NO. 299	SEQ.ID.NO. 442	PLT00014333A16.contig.a
HG1015015	SEQ.ID.NO. 113	SEQ.ID.NO. 300		PLT00014333A16.contig.b
HG1015016	SEQ.ID.NO. 114	SEQ.ID.NO. 301	SEQ.ID.NO. 443	PLT00014333B03.contig.a
HG1015017	SEQ.ID.NO. 115	SEQ.ID.NO. 302		PLT00014333B03.contig.b
HG1015018	SEQ.ID.NO. 116	SEQ.ID.NO. 303	SEQ.ID.NO. 444	PLT00014333B05.contig.a
HG1015019	SEQ.ID.NO. 117	SEQ.ID.NO. 304		PLT00014333B05.contig.b
HG1015020	SEQ.ID.NO. 118	SEQ.ID.NO. 305	SEQ.ID.NO. 445	PLT00014333B15.contig.a
HG1015021	SEQ.ID.NO. 119	SEQ.ID.NO. 306	SEQ.ID.NO. 446	PLT00014333B17.contig.a
HG1015022	SEQ.ID.NO. 120	SEQ.ID.NO. 307		PLT00014333B17.contig.b
HG1015023	SEQ.ID.NO. 121	SEQ.ID.NO. 308	SEQ.ID.NO. 447	PLT00014333C02.contig.a
HG1015024	SEQ.ID.NO. 122	SEQ.ID.NO. 309		PLT00014333C02.contig.b
HG1015025	SEQ.ID.NO. 123	SEQ.ID.NO. 310	SEQ.ID.NO. 448	PLT00014333C10.contig.a
HG1015026	SEQ.ID.NO. 124	SEQ.ID.NO. 311		PLT00014333C10.contig.b
HG1015027	SEQ.ID.NO. 125	SEQ.ID.NO. 312	SEQ.ID.NO. 449	PLT00014333C16.contig.a
HG1015028	SEQ.ID.NO. 126	SEQ.ID.NO. 313		PLT00014333C16.contig.b
HG1015029	SEQ.ID.NO. 127	SEQ.ID.NO. 314	SEQ.ID.NO. 450	PLT00014333C21.contig.a
HG1015030	SEQ.ID.NO. 128	SEQ.ID.NO. 315		PLT00014333C21.contig.b
HG1015031	SEQ.ID.NO. 129	SEQ.ID.NO. 316	SEQ.ID.NO. 451	PLT00014333C24.contig.a
HG1015032	SEQ.ID.NO. 130	SEQ.ID.NO. 317		PLT00014333C24.contig.b
HG1015033	SEQ.ID.NO. 131	SEQ.ID.NO. 318	SEQ.ID.NO. 452	PLT00014333D07.contig.a
HG1015034	SEQ.ID.NO. 132	SEQ.ID.NO. 319		PLT00014333D07.contig.b
HG1015035	SEQ.ID.NO. 133	SEQ.ID.NO. 320	SEQ.ID.NO. 453	PLT00014333D15.contig.a
HG1015036	SEQ.ID.NO. 134	SEQ.ID.NO. 321		PLT00014333D15.contig.b
HG1015037	SEQ.ID.NO. 135	SEQ.ID.NO. 322	SEQ.ID.NO. 454	PLT00014333E01.contig.a
HG1015038	SEQ.ID.NO. 136	SEQ.ID.NO. 323		PLT00014333E01.contig.b
HG1015039	SEQ.ID.NO. 137	SEQ.ID.NO. 324	SEQ.ID.NO. 455	PLT00014333E04.contig.a
HG1015040	SEQ.ID.NO. 138	SEQ.ID.NO. 325	SEQ.ID.NO. 456	PLT00014333E05.contig.a
HG1015041	SEQ.ID.NO. 139	SEQ.ID.NO. 326		PLT00014333E05.contig.b
HG1015042	SEQ.ID.NO. 140	SEQ.ID.NO. 327	SEQ.ID.NO. 457	PLT00014333E14.contig.a
HG1015043	SEQ.ID.NO. 141	SEQ.ID.NO. 328		PLT00014333E14.contig.b
HG1015086	SEQ.ID.NO. 142	SEQ.ID.NO. 329	SEQ.ID.NO. 458	PLT00014333E15.contig.a
HG1015087	SEQ.ID.NO. 143	SEQ.ID.NO. 330		PLT00014333E15.contig.b
HG1015044	SEQ.ID.NO. 144	SEQ.ID.NO. 331	SEQ.ID.NO. 459	PLT00014333E24.contig.b
HG1015045	SEQ.ID.NO. 145	SEQ.ID.NO. 332	SEQ.ID.NO. 460	PLT00014333F07.contig.a
HG1015046	SEQ.ID.NO. 146	SEQ.ID.NO. 333	SEQ.ID.NO. 461	PLT00014333G01.contig.a
HG1015047	SEQ.ID.NO. 147	SEQ.ID.NO. 334	SEQ.ID.NO. 462	PLT00014333G02.contig.a
HG1015048	SEQ.ID.NO. 148	SEQ.ID.NO. 335		PLT00014333G02.contig.b
HG1015088	SEQ.ID.NO. 149	SEQ.ID.NO. 336	SEQ.ID.NO. 463	PLT00014333G09.contig.a
HG1015089	SEQ.ID.NO. 150	SEQ.ID.NO. 337		PLT00014333G09.contig.b
HG1015049	SEQ.ID.NO. 151	SEQ.ID.NO. 338	SEQ.ID.NO. 464	PLT00014333H11.contig.a
HG1015050	SEQ.ID.NO. 152	SEQ.ID.NO. 339	SEQ.ID.NO. 465	PLT00014333H15.contig.a
HG1015051	SEQ.ID.NO. 153	SEQ.ID.NO. 340		PLT00014333H15.contig.b

FP ID	SEQ.ID.NO. (N1)	SEQ.ID.NO. (P1)	SEQ.ID.NO. (N0)	Clone ID
HG1015052	SEQ.ID.NO. 154	SEQ.ID.NO. 341	SEQ.ID.NO. 466	PLT00014333I18.contig.a
HG1015053	SEQ.ID.NO. 155	SEQ.ID.NO. 342		PLT00014333I18.contig.b
HG1015054	SEQ.ID.NO. 156	SEQ.ID.NO. 343	SEQ.ID.NO. 467	PLT00014333I22.contig.a
HG1015055	SEQ.ID.NO. 157	SEQ.ID.NO. 344		PLT00014333I22.contig.b
HG1015056	SEQ.ID.NO. 158	SEQ.ID.NO. 345	SEQ.ID.NO. 468	PLT00014333J01.contig.a
HG1015057	SEQ.ID.NO. 159	SEQ.ID.NO. 346		PLT00014333J01.contig.b
HG1015058	SEQ.ID.NO. 160	SEQ.ID.NO. 347	SEQ.ID.NO. 469	PLT00014333J13.contig.a
HG1015059	SEQ.ID.NO. 161	SEQ.ID.NO. 348		PLT00014333J13.contig.b
HG1015060	SEQ.ID.NO. 162	SEQ.ID.NO. 349	SEQ.ID.NO. 470	PLT00014333J15.contig.a
HG1015061	SEQ.ID.NO. 163	SEQ.ID.NO. 350		PLT00014333J15.contig.b
HG1015062	SEQ.ID.NO. 164	SEQ.ID.NO. 351	SEQ.ID.NO. 471	PLT00014333J17.contig.a
HG1015063	SEQ.ID.NO. 165	SEQ.ID.NO. 352	SEQ.ID.NO. 472	PLT00014333J23.contig.a
HG1015064	SEQ.ID.NO. 166	SEQ.ID.NO. 353		PLT00014333J23.contig.b
HG1015065	SEQ.ID.NO. 167	SEQ.ID.NO. 354	SEQ.ID.NO. 473	PLT00014333K04.contig.a
HG1015066	SEQ.ID.NO. 168	SEQ.ID.NO. 355		PLT00014333K04.contig.b
HG1015067	SEQ.ID.NO. 169	SEQ.ID.NO. 356	SEQ.ID.NO. 474	PLT00014333K08.contig.a
HG1015068	SEQ.ID.NO. 170	SEQ.ID.NO. 357		PLT00014333K08.contig.b
HG1015069	SEQ.ID.NO. 171	SEQ.ID.NO. 358	SEQ.ID.NO. 475	PLT00014333L13.contig.b
HG1015070	SEQ.ID.NO. 172	SEQ.ID.NO. 359	SEQ.ID.NO. 476	PLT00014333M01.contig.a
HG1015071	SEQ.ID.NO. 173	SEQ.ID.NO. 360		PLT00014333M01.contig.b
HG1015072	SEQ.ID.NO. 174	SEQ.ID.NO. 361	SEQ.ID.NO. 477	PLT00014333M02.contig.a
HG1015073	SEQ.ID.NO. 175	SEQ.ID.NO. 362		PLT00014333M02.contig.b
HG1015074	SEQ.ID.NO. 176	SEQ.ID.NO. 363	SEQ.ID.NO. 478	PLT00014333M07.contig.a
HG1015075	SEQ.ID.NO. 177	SEQ.ID.NO. 364		PLT00014333M07.contig.b
HG1015076	SEQ.ID.NO. 178	SEQ.ID.NO. 365	SEQ.ID.NO. 479	PLT00014333M15.contig.a
HG1015077	SEQ.ID.NO. 179	SEQ.ID.NO. 366		PLT00014333M15.contig.b
HG1015078	SEQ.ID.NO. 180	SEQ.ID.NO. 367	SEQ.ID.NO. 480	PLT00014333N05.contig.a
HG1015079	SEQ.ID.NO. 181	SEQ.ID.NO. 368		PLT00014333N05.contig.b
HG1015080	SEQ.ID.NO. 182	SEQ.ID.NO. 369	SEQ.ID.NO. 481	PLT00014333N11.contig.a
HG1015081	SEQ.ID.NO. 183	SEQ.ID.NO. 370		PLT00014333N11.contig.b
HG1015082	SEQ.ID.NO. 184	SEQ.ID.NO. 371	SEQ.ID.NO. 482	PLT00014333O03.contig.a
HG1015083	SEQ.ID.NO. 185	SEQ.ID.NO. 372		PLT00014333O03.contig.b
HG1015084	SEQ.ID.NO. 186	SEQ.ID.NO. 373	SEQ.ID.NO. 483	PLT00014333O10.contig.a
HG1015085	SEQ.ID.NO. 187	SEQ.ID.NO. 374	SEQ.ID.NO. 484	PLT00014333O17.contig.a

Table 2. Structural Characteristics

FP ID	Clone ID	Predicted Protein Length	Tree Vote	Mature Protein Coords.	Alternate Mature Protein Coords.	Signal Peptide Coords.	TM	TM Coords.	Non-TM Coords.	Pfam
HG1014903	PLT00014330A02.contig.a	89	0	(1-89)			0		(1-89)	no_pfam
HG1014904	PLT00014330A02.contig.b	87	0	(1-87)			0		(1-87)	no_pfam
HG1014905	PLT00014330A08.contig.a	82	0.55	(27-82)		(1-26)	1	(15-37)	(1-14)(38-82)	no_pfam
HG1014906	PLT00014330A08.contig.b	61	0.62	(24-61)		(6-23)	2	(5-27)(31-53)	(1-4)(28-30)(54-61)	no_pfam
HG1014907	PLT00014330A17.contig.a	66	0.11	(1-66)	(39-66)	(11-38)	0		(1-66)	no_pfam
HG1014908	PLT00014330A20.contig.a	54	0.25	(33-54)		(18-32)	0		(1-54)	no_pfam
HG1014909	PLT00014330B02.contig.a	84	0	(1-84)			0		(1-84)	no_pfam
HG1014910	PLT00014330B02.contig.b	73	0.07	(22-73)	(41-73)	(16-40)	0		(1-73)	no_pfam
HG1014911	PLT00014330B04.contig.a	160	0	(1-160)			0		(1-160)	no_pfam
HG1014912	PLT00014330B04.contig.b	108	0.05	(1-108)	(25-108)	(11-24)	0		(1-108)	no_pfam
HG1014913	PLT00014330B05.contig.a	79	0.02	(1-79)			0		(1-79)	no_pfam
HG1014914	PLT00014330B11.contig.a	68	0.23	(15-68)	(26-68)	(1-25)	0		(1-68)	no_pfam
HG1014915	PLT00014330B13.contig.a	55	0.05	(1-55)	(38-55)	(8-37)	0		(1-55)	no_pfam
HG1014916	PLT00014330B13.contig.b	53	0.01	(1-53)	(20-53)	(1-19)	0		(1-53)	no_pfam
HG1014917	PLT00014330B18.contig.a	74	0.7	(22-74)		(2-21)	0		(1-74)	no_pfam
HG1014918	PLT00014330B18.contig.b	53	0.24	(28-53)	(37-53)	(14-36)	0		(1-53)	no_pfam
HG1014919	PLT00014330C06.contig.a	101	0.53	(20-101)	(44-101)	(19-43)	0		(1-101)	no_pfam
HG1014920	PLT00014330C06.contig.b	65	0.01	(1-65)	(18-65)	(1-17)	0		(1-65)	no_pfam
HG1014921	PLT00014330C12.contig.a	68	0.01	(1-68)	(23-68)	(1-22)	0		(1-68)	no_pfam
HG1014922	PLT00014330C14.contig.a	66	0.02	(1-66)			0		(1-66)	no_pfam
HG1014923	PLT00014330C18.contig.a	64	0	(1-64)	(20-64)	(1-19)	0		(1-64)	no_pfam
HG1014924	PLT00014330C18.contig.b	63	0	(1-63)			0		(1-63)	no_pfam
HG1014925	PLT00014330D03.contig.a	132	0.81	(20-132)		(1-19)	0		(1-132)	no_pfam
HG1014926	PLT00014330D03.contig.b	74	0.43	(37-74)		(15-36)	2	(12-31)(46-68)	(1-11)(32-45)(69-74)	no_pfam



FP ID	Clone ID	Predicted Protein Length	Tree Vote	Mature Protein Coords.	Alternate Mature Protein Coords.	Signal Peptide Coords.	TM	TM Coords.	Non-TM Coords.	Pfam
HG1014927	PLT00014330D05.contig.a	60	0.07	(1-60)	(32-60)	(16-31)	0		(1-60)	no_pfam
HG1014928	PLT00014330D05.contig.b	54	0.39	(1-54)	(27-54)	(1-26)	0		(1-54)	no_pfam
HG1014929	PLT00014330D07.contig.a	85	0.03	(4-85)	(1-85)		0		(1-85)	no_pfam
HG1014930	PLT00014330D10.contig.a	79	0.61	(29-79)	(30-79)	(6-29)	0		(1-79)	no_pfam
HG1014931	PLT00014330D10.contig.b	73	0.87	(22-73)	(20-73)	(1-19)	0		(1-73)	no_pfam
HG1014932	PLT00014330D12.contig.a	116	0.01	(1-116)			1	(21-43)	(1-20)(44-116)	no_pfam
HG1014933	PLT00014330D12.contig.b	54	0.24	(24-54)		(1-23)	0		(1-54)	no_pfam
HG1014934	PLT00014330D13.contig.a	60	0	(1-60)			0		(1-60)	no_pfam
HG1014935	PLT00014330D15.contig.a	92	0.01	(1-92)	(21-92)	(6-20)	0		(1-92)	no_pfam
HG1014936	PLT00014330D15.contig.b	89	0.4	(36-89)	(46-89)	(16-45)	1	(12-34)	(1-11)(35-89)	no_pfam
HG1014937	PLT00014330D17.contig.a	96	0.26	(30-96)	(27-96)	(10-26)	0		(1-96)	no_pfam
HG1014938	PLT00014330E04.contig.a	54	0.02	(1-54)			0		(1-54)	no_pfam
HG1014939	PLT00014330E14.contig.a	68	0.02	(1-68)	(19-68)	(1-18)	0		(1-68)	no_pfam
HG1014940	PLT00014330E14.contig.b	61	0	(1-61)	(27-61)	(9-26)	0		(1-61)	no_pfam
HG1014941	PLT00014330E24.contig.a	112	0.01	(1-112)			0		(1-112)	no_pfam
HG1014942	PLT00014330E24.contig.b	62	0.16	(1-62)	(35-62)	(17-34)	1	(15-34)	(1-14)(35-62)	no_pfam
HG1014943	PLT00014330F01.contig.a	77	0	(1-77)			1	(28-45)	(1-27)(46-77)	no_pfam
HG1014944	PLT00014330F03.contig.a	105	0	(1-105)			0		(1-105)	no_pfam
HG1014945	PLT00014330F03.contig.b	71	0.01	(27-71)	(1-71)		0		(1-71)	no_pfam
HG1014946	PLT00014330F04.contig.a	117	0.9	(18-117)	(20-117)	(1-19)	0		(1-117)	no_pfam
HG1014947	PLT00014330F04.contig.b	104	0.09	(25-104)		(1-24)	0		(1-104)	no_pfam
HG1014948	PLT00014330F05.contig.a	50	0.01	(1-50)	(16-50)	(1-15)	0		(1-50)	no_pfam
HG1014949	PLT00014330F13.contig.a	53	0.26	(28-53)		(1-27)	0		(1-53)	no_pfam
HG1014950	PLT00014330G21.contig.a	146	0.16	(28-146)	(29-146)	(6-28)	0		(1-146)	no_pfam
HG1014951	PLT00014330G21.contig.b	53	0.05	(1-53)			1	(20-42)	(1-19)(43-53)	no_pfam

FP ID	Clone ID	Predicted Protein Length	Tree Vote	Mature Protein Coords.	Alternate Mature Protein Coords.	Signal Peptide Coords.	TM	TM Coords.	Non-TM Coords.	Pfam
HG1014952	PLT00014330H05.contig.b	97	0.01	(1-97)	(25-97)	(1-24)	0		(1-97)	rvt
HG1014953	PLT00014330H06.contig.a	50	0.16	(1-50)	(32-50)	(16-31)	0		(1-50)	no_pfam
HG1014954	PLT00014330H12.contig.a	86	0.65	(19-86)		(1-18)	0		(1-86)	no_pfam
HG1014955	PLT00014330H12.contig.b	76	0.03	(1-76)	(19-76)	(1-18)	0		(1-76)	no_pfam
HG1014956	PLT00014330H14.contig.a	68	0.2	(38-68)	(17-68)	(1-16)	0		(1-68)	no_pfam
HG1014957	PLT00014330H14.contig.b	66	0.05	(29-66)	(1-66)		1	(43-62)	(1-42)(63-66)	no_pfam
HG1014958	PLT00014330H18.contig.a	95	0.94	(21-95)	(19-95)	(1-18)	0		(1-95)	no_pfam
HG1014959	PLT00014330H18.contig.b	77	0.01	(38-77)	(1-77)		0		(1-77)	no_pfam
HG1014960	PLT00014330I11.contig.a	62	0.05	(1-62)			1	(31-53)	(1-30)(54-62)	no_pfam
HG1014961	PLT00014330I12.contig.a	88	0.3	(8-88)	(19-88)	(1-18)	0		(1-88)	no_pfam
HG1014962	PLT00014330I12.contig.b	66	0.51	(8-66)	(16-66)	(1-15)	2	(4-26)(43-65)	(1-3)(27-42)(66-66)	no_pfam
HG1014963	PLT00014330I13.contig.a	103	0.04	(1-103)	(41-103)	(17-40)	0		(1-103)	no_pfam
HG1014964	PLT00014330I13.contig.b	84	0.02	(1-84)	(18-84)	(5-17)	0		(1-84)	no_pfam
HG1014965	PLT00014330J10.contig.a	130	0.05	(16-130)	(1-130)		0		(1-130)	no_pfam
HG1014966	PLT00014330J10.contig.b	103	0	(1-103)			0		(1-103)	no_pfam
HG1014967	PLT00014330J14.contig.a	79	0.02	(32-79)	(1-79)		0		(1-79)	no_pfam
HG1014968	PLT00014330J14.contig.b	57	0.03	(1-57)	(23-57)	(1-22)	0		(1-57)	no_pfam
HG1014969	PLT00014330J15.contig.a	68	0.01	(1-68)			0		(1-68)	no_pfam
HG1014970	PLT00014330J21.contig.a	80	0.1	(1-80)	(25-80)	(10-24)	0		(1-80)	no_pfam
HG1014971	PLT00014330J21.contig.b	68	0.08	(1-68)	(22-68)	(1-21)	0		(1-68)	no_pfam
HG1014972	PLT00014330K01.contig.a	73	0	(1-73)			0		(1-73)	no_pfam
HG1014973	PLT00014330K08.contig.a	99	0.16	(1-99)	(26-99)	(1-25)	1	(73-95)	(1-72)(96-99)	no_pfam
HG1014974	PLT00014330K08.contig.b	50	0.26	(1-50)	(18-50)	(1-17)	2	(5-27)(32-49)	(1-4)(28-31)(50-50)	no_pfam
HG1014975	PLT00014330K09.contig.a	100	0.09	(20-100)		(2-19)	0		(1-100)	no_pfam
HG1014976	PLT00014330K09.contig.b	60	0	(1-60)	(23-60)	(11-22)	0		(1-60)	no_pfam
HG1014977	PLT00014330K15.contig.a	72	0.01	(1-72)	(26-72)	(2-25)	0		(1-72)	no_pfam
HG1014978	PLT00014330K15.contig.b	61	0	(1-61)	(33-61)	(9-32)	0		(1-61)	no_pfam

FP ID	Clone ID	Predicted Protein Length	Tree Vote	Mature Protein Coords.	Alternate Mature Protein Coords.	Signal Peptide Coords.	TM	TM Coords.	Non-TM Coords.	Pfam
HG1014979	PLT00014330K24.contig.a	51	0.17	(37-51)	(29-51)	(8-28)	1	(13-35)	(1-12)(36-51)	no_pfam
HG1014980	PLT00014330L01.contig.a	112	0.13	(37-112)	(19-112)	(1-18)	0		(1-112)	no_pfam
HG1014981	PLT00014330M02.contig.a	106	0.01	(1-106)			0		(1-106)	no_pfam
HG1014982	PLT00014330M02.contig.b	88	0.27	(1-88)	(19-88)	(1-18)	0		(1-88)	no_pfam
HG1014983	PLT00014330M08.contig.a	72	0.46	(32-72)		(18-31)	1	(45-67)	(1-44)(68-72)	no_pfam
HG1014984	PLT00014330M08.contig.b	52	0.29	(31-52)		(17-30)	1	(20-42)	(1-19)(43-52)	no_pfam
HG1014985	PLT00014330M15.contig.a	53	0.07	(1-53)	(53-53)	(19-52)	0		(1-53)	no_pfam
HG1014986	PLT00014330M17.contig.a	110	0.13	(1-110)	(21-110)	(1-20)	0		(1-110)	no_pfam
HG1014987	PLT00014330M17.contig.b	82	0.45	(29-82)	(30-82)	(16-29)	0		(1-82)	no_pfam
HG1014988	PLT00014330N10.contig.a	75	0.15	(38-75)		(18-37)	1	(20-42)	(1-19)(43-75)	no_pfam
HG1014989	PLT00014330N10.contig.b	68	0	(1-68)	(22-68)	(1-21)	0		(1-68)	no_pfam
HG1014990	PLT00014330N12.contig.a	56	0	(1-56)	(33-56)	(18-32)	0		(1-56)	no_pfam
HG1014991	PLT00014330N12.contig.b	56	0	(1-56)	(20-56)	(1-19)	0		(1-56)	no_pfam
HG1014992	PLT00014330N13.contig.a	83	0.87	(23-83)	(20-83)	(1-19)	1	(4-26)	(1-3)(27-83)	no_pfam
HG1014993	PLT00014330N13.contig.b	55	0.29	(28-55)	(29-55)	(14-28)	1	(10-32)	(1-9)(33-55)	no_pfam
HG1014994	PLT00014330N22.contig.a	74	0.02	(1-74)	(33-74)	(19-32)	0		(1-74)	no_pfam
HG1014995	PLT00014330N22.contig.b	57	0.12	(1-57)	(20-57)	(1-19)	0		(1-57)	no_pfam
HG1014996	PLT00014330O03.contig.a	70	0.32	(1-70)	(19-70)	(5-18)	1	(7-29)	(1-6)(30-70)	no_pfam
HG1014997	PLT00014330O07.contig.a	78	0	(1-78)			0		(1-78)	no_pfam
HG1014998	PLT00014330O07.contig.b	73	0.06	(1-73)	(33-73)	(19-32)	0		(1-73)	no_pfam
HG1014999	PLT00014330P07.contig.a	85	0.03	(1-85)	(33-85)	(1-32)	0		(1-85)	no_pfam
HG1015000	PLT00014330P07.contig.b	61	0.05	(34-61)	(32-61)	(1-31)	0		(1-61)	no_pfam
HG1015001	PLT00014330P09.contig.a	101	0.17	(1-101)	(33-101)	(13-32)	0		(1-101)	no_pfam
HG1015002	PLT00014330P09.contig.b	98	0.01	(1-98)			0		(1-98)	no_pfam
HG1015003	PLT00014330P15.contig.a	61	0.02	(1-61)			0		(1-61)	no_pfam

FP ID	Clone ID	Predicted Protein Length	Tree Vote	Mature Protein Coords.	Alternate Mature Protein Coords.	Signal Peptide Coords.	TM	TM Coords.	Non-TM Coords.	Pfam
HG1015004	PLT00014330L24.contig.a	50	0.17	(38-50)	(34-50)	(1-33)	0		(1-50)	no_pfam
HG1015005	PLT00014330O18.contig.a	82	0	(1-82)			0		(1-82)	no_pfam
HG1015006	PLT00014330O18.contig.b	66	0	(1-66)			0		(1-66)	no_pfam
HG1015007	PLT00014333A03.contig.a	83	0.08	(1-83)	(39-83)	(19-38)	1	(15-37)	(1-14)(38-83)	no_pfam
HG1015008	PLT00014333A03.contig.b	64	0.1	(30-64)	(29-64)	(11-28)	0		(1-64)	no_pfam
HG1015009	PLT00014333A06.contig.a	153	0.01	(1-153)			0		(1-153)	no_pfam
HG1015010	PLT00014333A06.contig.b	66	0.13	(35-66)	(33-66)	(18-32)	0		(1-66)	no_pfam
HG1015011	PLT00014333A08.contig.a	66	0.26	(1-66)	(22-66)	(1-21)	0		(1-66)	no_pfam
HG1015012	PLT00014333A15.contig.a	136	0.03	(1-136)			0		(1-136)	no_pfam
HG1015013	PLT00014333A15.contig.b	67	0.8	(38-67)	(35-67)	(17-34)	0		(1-67)	no_pfam
HG1015014	PLT00014333A16.contig.a	51	0.02	(1-51)			0		(1-51)	no_pfam
HG1015015	PLT00014333A16.contig.b	50	0.46	(25-50)	(41-50)	(16-40)	0		(1-50)	no_pfam
HG1015016	PLT00014333B03.contig.a	63	0.02	(1-63)			0		(1-63)	no_pfam
HG1015017	PLT00014333B03.contig.b	50	0	(1-50)	(15-50)	(1-14)	0		(1-50)	no_pfam
HG1015018	PLT00014333B05.contig.a	55	0.05	(1-55)			1	(29-51)	(1-28)(52-55)	no_pfam
HG1015019	PLT00014333B05.contig.b	53	0.49	(1-53)	(18-53)	(1-17)	0		(1-53)	no_pfam
HG1015020	PLT00014333B15.contig.a	53	0	(1-53)	(28-53)	(3-27)	0		(1-53)	no_pfam
HG1015021	PLT00014333B17.contig.a	76	0.35	(16-76)		(1-15)	0		(1-76)	no_pfam
HG1015022	PLT00014333B17.contig.b	65	0.01	(1-65)			1	(42-64)	(1-41)(65-65)	no_pfam
HG1015023	PLT00014333C02.contig.a	77	0.03	(1-77)			0		(1-77)	no_pfam
HG1015024	PLT00014333C02.contig.b	51	0.77	(22-51)		(8-21)	1	(12-34)	(1-11)(35-51)	no_pfam
HG1015025	PLT00014333C10.contig.a	99	0.33	(1-99)	(50-99)	(19-49)	0		(1-99)	no_pfam
HG1015026	PLT00014333C10.contig.b	92	0.21	(18-92)	(20-92)	(1-19)	0		(1-92)	no_pfam
HG1015027	PLT00014333C16.contig.a	363	0.04	(1-363)	(15-363)	(1-14)	0		(1-363)	no_pfam

FP ID	Clone ID	Predicted Protein Length	Tree Vote	Mature Protein Coords.	Alternate Mature Protein Coords.	Signal Peptide Coords.	TM	TM Coords.	Non-TM Coords.	Pfam
HG1015028	PLT00014333C16.contig.b	86	0.24	(1-86)	(27-86)	(1-26)	0		(1-86)	no_pfam
HG1015029	PLT00014333C21.contig.a	82	0.49	(1-82)	(49-82)	(19-48)	0		(1-82)	no_pfam
HG1015030	PLT00014333C21.contig.b	77	0.03	(1-77)	(28-77)	(9-27)	0		(1-77)	no_pfam
HG1015031	PLT00014333C24.contig.a	94	0.11	(1-94)	(30-94)	(15-29)	1	(10-32)	(1-9)(33-94)	no_pfam
HG1015032	PLT00014333C24.contig.b	88	0	(1-88)			2	(34-56)(61-78)	(1-33)(57-60)(79-88)	no_pfam
HG1015033	PLT00014333D07.contig.a	73	0.02	(1-73)	(21-73)	(1-20)	0		(1-73)	no_pfam
HG1015034	PLT00014333D07.contig.b	67	0.23	(1-67)	(32-67)	(1-31)	0		(1-67)	no_pfam
HG1015035	PLT00014333D15.contig.a	64	0.11	(32-64)	(31-64)	(16-30)	0		(1-64)	no_pfam
HG1015036	PLT00014333D15.contig.b	62	0.29	(34-62)	(31-62)	(5-30)	2	(13-32)(42-61)	(1-12)(33-41)(62-62)	no_pfam
HG1015037	PLT00014333E01.contig.a	73	0	(36-73)	(1-73)		1	(26-48)	(1-25)(49-73)	no_pfam
HG1015038	PLT00014333E01.contig.b	67	0.51	(35-67)	(26-67)	(8-25)	1	(10-32)	(1-9)(33-67)	no_pfam
HG1015039	PLT00014333E04.contig.a	53	0.01	(1-53)			0		(1-53)	no_pfam
HG1015040	PLT00014333E05.contig.a	66	0.01	(1-66)	(25-66)	(8-24)	0		(1-66)	no_pfam
HG1015041	PLT00014333E05.contig.b	57	0.03	(1-57)	(45-57)	(1-44)	0		(1-57)	no_pfam
HG1015042	PLT00014333E14.contig.a	108	0.01	(1-108)			0		(1-108)	no_pfam
HG1015043	PLT00014333E14.contig.b	61	0.24	(26-61)	(29-61)	(14-28)	0		(1-61)	no_pfam
HG1015044	PLT00014333E24.contig.b	91	0.01	(1-91)	(32-91)	(18-31)	0		(1-91)	Transposase_1
HG1015045	PLT00014333F07.contig.a	52	0	(1-52)	(17-52)	(1-16)	0		(1-52)	no_pfam
HG1015046	PLT00014333G01.contig.a	69	0.24	(1-69)	(33-69)	(14-32)	0		(1-69)	no_pfam
HG1015047	PLT00014333G02.contig.a	77	0.03	(19-77)	(1-77)		0		(1-77)	no_pfam
HG1015048	PLT00014333G02.contig.b	57	0	(1-57)			0		(1-57)	no_pfam
HG1015049	PLT00014333H11.contig.a	95	0.03	(1-95)	(36-95)	(12-35)	0		(1-95)	no_pfam
HG1015050	PLT00014333H15.contig.a	90	0.23	(35-90)		(1-34)	0		(1-90)	no_pfam
HG1015051	PLT00014333H15.contig.b	60	0	(1-60)			0		(1-60)	no_pfam
HG1015052	PLT00014333I18.contig.a	58	0.69	(22-58)	(34-58)	(12-33)	1	(7-29)	(1-6)(30-58)	no_pfam
HG1015053	PLT00014333I18.contig.b	50	0.77	(22-50)		(1-21)	0		(1-50)	no_pfam
HG1015054	PLT00014333I22.contig.a	70	0.08	(1-70)	(19-70)	(1-18)	0		(1-70)	no_pfam

FP ID	Clone ID	Predicted Protein Length	Tree Vote	Mature Protein Coords.	Alternate Mature Protein Coords.	Signal Peptide Coords.	TM	TM Coords.	Non-TM Coords.	Pfam
HG1015055	PLT00014333J22.contig.b	54	0.96	(23-54)	(25-54)	(1-24)	1	(6-28)	(1-5)(29-54)	no_pfam
HG1015056	PLT00014333J01.contig.a	84	0.03	(1-84)	(35-84)	(19-34)	0		(1-84)	no_pfam
HG1015057	PLT00014333J01.contig.b	66	0.08	(32-66)	(33-66)	(1-32)	0		(1-66)	no_pfam
HG1015058	PLT00014333J13.contig.a	106	0.02	(1-106)			1	(46-68)	(1-45)(69-106)	no_pfam
HG1015059	PLT00014333J13.contig.b	93	0.06	(37-93)	(1-93)		0		(1-93)	no_pfam
HG1015060	PLT00014333J15.contig.a	63	0.12	(1-63)	(17-63)	(1-16)	0		(1-63)	no_pfam
HG1015061	PLT00014333J15.contig.b	62	0.18	(1-62)	(22-62)	(7-21)	1	(20-42)	(1-19)(43-62)	no_pfam
HG1015062	PLT00014333J17.contig.a	88	0	(1-88)	(36-88)	(16-35)	0		(1-88)	no_pfam
HG1015063	PLT00014333J23.contig.a	66	0.05	(1-66)	(16-66)	(1-15)	0		(1-66)	no_pfam
HG1015064	PLT00014333J23.contig.b	57	0.33	(1-57)	(31-57)	(14-30)	0		(1-57)	no_pfam
HG1015065	PLT00014333K04.contig.a	131	0.01	(1-131)			0		(1-131)	Gag_p24
HG1015066	PLT00014333K04.contig.b	125	0.14	(1-125)	(19-125)	(1-18)	0		(1-125)	integrase
HG1015067	PLT00014333K08.contig.a	69	0.19	(1-69)	(34-69)	(19-33)	1	(28-50)	(1-27)(51-69)	no_pfam
HG1015068	PLT00014333K08.contig.b	63	0.17	(21-63)		(1-20)	0		(1-63)	no_pfam
HG1015069	PLT00014333L13.contig.b	52	0	(1-52)			0		(1-52)	maseH
HG1015070	PLT00014333M01.contig.a	110	0.29	(1-110)	(20-110)	(1-19)	1	(86-108)	(1-85)(109-110)	no_pfam
HG1015071	PLT00014333M01.contig.b	68	0.01	(1-68)	(18-68)	(1-17)	1	(41-63)	(1-40)(64-68)	no_pfam
HG1015072	PLT00014333M02.contig.a	101	0.01	(38-101)	(43-101)	(12-42)	0		(1-101)	no_pfam
HG1015073	PLT00014333M02.contig.b	50	0	(1-50)	(14-50)	(1-13)	0		(1-50)	no_pfam
HG1015074	PLT00014333M07.contig.a	70	0.26	(37-70)	(30-70)	(4-29)	1	(13-35)	(1-12)(36-70)	no_pfam
HG1015075	PLT00014333M07.contig.b	58	0.62	(15-58)	(16-58)	(1-15)	0		(1-58)	no_pfam
HG1015076	PLT00014333M15.contig.a	80	0.04	(1-80)	(42-80)	(18-41)	0		(1-80)	no_pfam
HG1015077	PLT00014333M15.contig.b	54	0.08	(1-54)	(42-54)	(18-41)	0		(1-54)	no_pfam
HG1015078	PLT00014333N05.contig.a	73	0.1	(5-73)	(15-73)	(1-14)	0		(1-73)	no_pfam
HG1015079	PLT00014333N05.contig.b	70	0.45	(35-70)	(39-70)	(5-38)	0		(1-70)	no_pfam

FP ID	Clone ID	Predicted Protein Length	Tree Vote	Mature Protein Coords.	Alternate Mature Protein Coords.	Signal Peptide Coords.	TM	TM Coords.	Non-TM Coords.	Pfam
HG1015080	PLT00014333N11.contig.a	95	0.01	(1-95)	(30-95)	(15-29)	0		(1-95)	no_pfam
HG1015081	PLT00014333N11.contig.b	69	0.03	(9-69)	(22-69)	(5-21)	0		(1-69)	no_pfam
HG1015082	PLT00014333O03.contig.a	72	0.21	(3-72)	(28-72)	(14-27)	0		(1-72)	no_pfam
HG1015083	PLT00014333O03.contig.b	55	0.01	(1-55)	(25-55)	(10-24)	0		(1-55)	no_pfam
HG1015084	PLT00014333O10.contig.a	55	0.06	(4-55)	(15-55)	(1-14)	0		(1-55)	no_pfam
HG1015085	PLT00014333O17.contig.a	71	0.11	(1-71)	(20-71)	(1-19)	0		(1-71)	no_pfam
HG1015086	PLT00014333E15.contig.a	92	0.49	(20-92)		(1-19)	1	(5-27)	(1-4)(28-92)	no_pfam
HG1015087	PLT00014333E15.contig.b	78	0.01	(1-78)			1	(52-71)	(1-51)(72-78)	no_pfam
HG1015088	PLT00014333G09.contig.a	125	0	(1-125)			0		(1-125)	no_pfam
HG1015089	PLT00014333G09.contig.b	63	0.11	(1-63)	(41-63)	(18-40)	0		(1-63)	no_pfam

Table 3. Similarity to Known Sequences

FP ID	Clone ID	Top Hit Accession ID	Top Hit Annotation	Top Hit % ID	Top Human Hit Accession ID	Top Human Hit Annot	Top Human Hit % ID
HG1014903	PLT00014330A02.contig.a	gi 34529187 dbj BAC85656.1	unnamed protein product [Homo sapiens]	59	gi 34529187 dbj BAC85656.1	unnamed protein product [Homo sapiens]	59
HG1014910	PLT00014330B02.contig.b	gi 7770237 gb AAAF69654.1	PRO2822 [Homo sapiens]	76	gi 7770237 gb AAAF69654.1	PRO2822 [Homo sapiens]	76
HG1014914	PLT00014330B11.contig.a	gi 38085361 ref XP_355822.1	similar to RIKEN cDNA 6330419J24 gene [Mus musculus]	80		no_human_hit	
HG1014933	PLT00014330D12.contig.b	gi 8923214 ref NP_060190.1	signal-transducing adaptor protein-2; brk kinase substrate [Homo sapiens] gi 7020193 dbj BAA91028.1  unnamed protein product [Homo sapiens]	57	gi 8923214 ref NP_060190.1	signal-transducing adaptor protein-2; brk kinase substrate [Homo sapiens] gi 7020193 dbj BAA91028.1  unnamed protein product [Homo sapiens]	57
HG1014948	PLT00014330F05.contig.a	gi 34534372 dbj BAC86987.1	unnamed protein product [Homo sapiens]	56	gi 34534372 dbj BAC86987.1	unnamed protein product [Homo sapiens]	56



FP ID	Clone ID	Top Hit Accession ID	Top Hit Annotation	Top Hit % ID	Top Human Hit Accession ID	Top Human Hit Annot	Top Human Hit % ID
HG1014952	PLT00014330H05.contig.b	gi 2981631 d bj BAA25253.1	ORF2 [Canis familiaris]	58	no_human_hit		
HG1014958	PLT00014330H18.contig.a	gi 13310191  gb AAK18189.1	recombinant envelope protein [multiple sclerosis associated retrovirus element]	52	no_human_hit		
HG1014971	PLT00014330J21.contig.b	gi 23503335  ef NP_694983.1	hypothetical protein FLJ25952 [Homo sapiens] gi 21758947 dbj BAC05422.1  unnamed protein product [Homo sapiens]	64	gi 23503335  f NP_694983.1	hypothetical protein FLJ25952 [Homo sapiens] gi 21758947 dbj BAC05422.1  unnamed protein product [Homo sapiens]	64
HG1014975	PLT00014330K09.contig.a	gi 34528691  dbj BAC85556.1	unnamed protein product [Homo sapiens]	56	gi 34528691  j BAC85556.1	unnamed protein product [Homo sapiens]	56
HG1014977	PLT00014330K15.contig.a	gi 34533624  dbj BAC86755.1	unnamed protein product [Homo sapiens]	81	gi 34533624  j BAC86755.1	unnamed protein product [Homo sapiens]	81
HG1014983	PLT00014330M08.contig.a	gi 21754422  dbj BAC04501.1	unnamed protein product [Homo sapiens]	55	gi 21754422  j BAC04501.1	unnamed protein product [Homo sapiens]	55
HG1014992	PLT00014330N13.contig.a	gi 37182643  gb AAQ89122.1	DRDL5813 [Homo sapiens]	56	gi 37182643  j AAQ89122.1	DRDL5813 [Homo sapiens]	56

FP ID	Clone ID	Top Hit Accession ID	Top Hit Annotation	Top Hit % ID	Top Human Hit Accession ID	Top Human Hit Annot	Top Human Hit % ID
HG1015030	PLT00014333C21.contig.b	gi 18027736 gb AAL55829.1	unknown [Homo sapiens]	87	gi 18027736 gb AAL55829.1	unknown [Homo sapiens]	87
HG1015044	PLT00014333E24.contig.b	gi 1698455 gb AAC52011.1	mariner transposase [Homo sapiens]	79	gi 1698455 gb AAC52011.1	mariner transposase [Homo sapiens]	79
HG1015082	PLT00014333O03.contig.a	gi 21754422 dbj BAC04501.1	unnamed protein product [Homo sapiens]	75	gi 21754422 dbj BAC04501.1	unnamed protein product [Homo sapiens]	75

# Appendix A

SEQ.ID.NO. 1 HG1014903N1      PLT00014330A02.contig.a  
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 TGCAGCCGTAAAGGGAACAAGATAATGTTCTTTTGCAAGGACGTGGGTGAGCTGGAAGC  
 CATTATCCTCAGCAAACTAACACAGGAACAGAAAACCAAACACACATGTTCTCACTTAT  
 AAGTGGGAGCCCAACAATGAGAACACATAG

SEQ.ID.NO. 2 HG1014904N1      PLT00014330A02.contig.b  
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 AGCAAACTAACACAGGAACAGAAAACCAAACACACATGTTCTCACTTATAAGTGGGAGC  
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SEQ.ID.NO. 6 HG1014908N1      PLT00014330A20.contig.a  
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SEQ.ID.NO. 13      HG1014915N1      PLT00014330B13.contig.a  
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 GGTTCACCTCAATCCCCCTTACTCAAGAACCTCAGTTCTACTGA

SEQ.ID.NO. 16      HG1014918N1      PLT00014330B18.contig.b  
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TCGATGGTCAAATTTTCTCTTACCAAGAGCTGGCCAGGTGCAGTGGCTCACACCTGTA  
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SEQ.ID.NO. 20      HG1014922N1      PLT00014330C14.contig.a  
ATGCAATTATCAAAACAAACCCAGTCAACCAGGATTGAAAGTGTCAAACAGTGCAACAATC  
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AGATTGATGAAAGATTGTCAGGTGACTTTTGGTCTCTCCTACATAGTCTGGAAATCAGA  
ATTGGAGCTTTAAACTTTAA



SEQ.ID.NO. 21     HG1014923N1     PLT00014330C18.contig.a  
 ATGCTGGGCAGCGTGTCCAGCCCCCTGCCTCGGGGTGGAAGGCAGAGAGCAAAGAGGA  
 GGCCCTCTTATTCGGACCCCCAGGCCAGAGCCCGAGGTGGAAAGCAGAGGGCAAAG  
 AGGAGGCCCCCTCCTCATCCCTGACCCCCAGGCCAGAGCCTAAGGTTCAAGTGCCTCAG  
 GCCCAGTCCCCCTTGA

SEQ.ID.NO. 22     HG1014924N1     PLT00014330C18.contig.b  
 ATGAGCACACTGAGCTCTGCAGGGCCTCAGCTGGAGGGCCATCCAATCGTCCTTCCCC  
 TCCCCAGCAGAACTCCAGAGGTGGGGCGGTGCCGAGGGCCCCAAGGCTGCCCTCGGCAGG  
 GGGCTTTGTGCTTTTGTGTGGAGGCCACCAAGGATTACAGGAAGATCCTGAATGGGTTG  
 TCAATTGACTGA

SEQ.ID.NO. 23     HG1014925N1     PLT00014330D03.contig.a  
 ATGACAAATACCCCGTCTCTGGTATCTTTGCACTTGCCCTCTTCAAAGCCACCTTTTCCTTT  
 CTCTCTCTGGACTGTCACAGATCCTCCTCTGTACCCCAAGAACACCCCAATTGGATTTC  
 CGGGCTGGCTGTGGCCTACACACCTGGGTCAAGGCCTCTCGCAGGGATGCGCCTGCCAC  
 TGTAATAAAGAGGAGAAACGTCAACAGGGAAGGCCTGACTCCTTCGTACAATCAGAAT  
 ACTCAACTGCACAGAGAGGGACCCCTTTAAGCCACTTTGGGAGCCACATCCACCCTCT  
 GTGACTCCACACAGGCTGGTTCACAGGTATCAGGTGTCTAGTGTAAACACGGGCCAA  
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SEQ.ID.NO. 24     HG1014926N1     PLT00014330D03.contig.b

ATGTGCATTTCTCTGCTGAGATTCCACATGTATTTCATTCATTATGAGAAATTTTCTT  
CACCCCATGACTATAGTTTTTAATAGCTGCTCTCAAATACTTGACTGCTGATAACAACATC  
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TTGTGTTTTCTCTCATGTCTCTTAAATTTTAAATTGTATTTTAA

SEQ.ID.NO. 25      HG1014927N1      PLT00014330D05.contig.a  
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ATGGACTTTAAAAAGAGGTCATCATAAGCAATAATATCCATGAAATCATGGATATCATG  
TGA

SEQ.ID.NO. 26      HG1014928N1      PLT00014330D05.contig.b  
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SEQ.ID.NO. 27      HG1014929N1      PLT00014330D07.contig.a  
ATGGTTTGTTTATACCTGATCTGTTTTCTGTATCAAAAATAAAATTAGGATGAATCTA  
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AGCATTTTGAGCTTTTCCATTATTGGTCCTTATACATTAAAGATTTTGTGGGGGTGGCA  
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SEQ.ID.NO. 28      HG1014930N1      PLT00014330D10.contig.a  
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 TCAAGCCATACACTGGCCTCGGCCTCCCTAAGTGCTGGGATTACAGGTACGAGCCACCGT  
 GCCTGGCCAGGAATCTGCATTTTGTCTAGTAACCCCTGGTCATCTCAGTGCACACCAAAGT  
 TTGAAACCACTGTTGGGGTGTGGAGTTCCTTGCTTCCAGACTTCCAGGGCCTATGGATTGA

SEQ.ID.NO. 29      HG1014931N1      PLT00014330D10.contig.b  
 ATGAACCTACTGCTGACCTTGGTTTATTTCGTTTGTGTTAATTTTCACCTTTTCTGTGAAA  
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 CCAGCTTCAGCAAAGATGACATTTTGCCACTCTTATCCATCCATTTCTGTCAAGCCACC  
 CCTGACATACACACATGTTCTTCTGGAGTATTTTCAAGC

SEQ.ID.NO. 30      HG1014932N1      PLT00014330D12.contig.a  
 ATGAGGAAGATGACATTTTGTCTGCAGATGGTGGAAATAAAAATCACAGAGATTGTGAT  
 TTCCTTATGGTTTTGTGGTAATGGTGGAGTTTAAACTTACAACGAAGTTTCTGGTGACA  
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 TCGCCCAGGCTGGAGTGCAGTGGTGATGTGTCGGCTCACTGCAACCTCTGCCCTCCTGG  
 ATTCAAGTATTCTCATGCGTCAGCCTCCAAGTAGCTGGGATTACAGGGGCCCAACCA  
 CGCCCAGCTAATTTTGTATTTTAGTATAGATGGAGTTTCACCATGTTGA

SEQ.ID.NO. 31      HG1014933N1      PLT00014330D12.contig.b  
 ATGGAGTTTCAACCATGTTGACCAAGGCTGGTCTTGAACCTCCTGGCCTCAAGTGACCCACCT  
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 TTCCTTATAATGATACAAGTAATATACCAGACAAGAAATTAC

SEQ.ID.NO. 32      HG1014934N1      PLT00014330D13.contig.a  
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 TTGATCCTAAATTTAATACAACAGAAAAAAGCAAGGATATTTTGACCAGTATAGATTTT  
 TAA

SEQ.ID.NO. 33      HG1014935N1      PLT00014330D15.contig.a  
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SEQ.ID.NO. 34      HG1014936N1      PLT00014330D15.contig.b  
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 CTCCTGGGTTCAAGAGATTCTCCTGCCTCAGCCTCCCGAGTAGCTGGGACTACAGGCACA

CGCCACCATGCCCAGCTAATTTTGTATTTTAGTAGAGATGGGGTTTCACCATGTTGGC  
CAGGATGGTCTTGTGATCTGTTGACCTCGTGA

SEQ.ID.NO. 35      HG1014937N1      PLT00014330D17.contig.a  
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ATGAGGGAGTGTATGCCTTTATACAAATTCACTCCAACCTTCAGAAAAACGTCCGCAGCTC  
ATGCTCCCCCTGCCAGAGCAGCAGTGTGAGCAGCTGTGTAGGTTTGGAAAGCACCCCAAGTC  
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SEQ.ID.NO. 36      HG1014938N1      PLT00014330E04.contig.a  
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CTGTACACTATCACCTTAAAGGACCTCCGAAAGTTGTTTCTGCTTATCTCTCCATAACCAA  
CTCCCTCCTTGCTGCACACTTCAGAAATTTTGGAAATAAGACATAA

SEQ.ID.NO. 37      HG1014939N1      PLT00014330E14.contig.a  
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CCCCTTCAACCAGCTACAGCAGGGCTGGCAATGCCCAAGTCCTTGGAGAAACAGAAAGAGAT  
TCAACTGCAACTGAAATTACCTACTAA

SEQ.ID.NO. 38      HG1014940N1      PLT00014330E14.contig.b  
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 GAATAA

SEQ.ID.NO. 39      HG1014941N1      PLT00014330E24.contig.a  
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 CCTGTGCAGCCTGGGAGAGCCCTGCTCACAGCCCACAGACAGGCTGCCTGTCTCACAC  
 CCTACTCAGGCCAGGAGCTGACCCCAAGAAATCACAGGGCTGGGCACAGCAGCCTCCCTGC  
 TCCCCACTGGCCCTGCTGTGAGTCACCTCACAGGTCTCTAGAGGACAAGGTTCTCACCTT  
 TCAAAATTATCTTTCTTACATATACACTTTACCCAAATGA

SEQ.ID.NO. 40      HG1014942N1      PLT00014330E24.contig.b  
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 AGAAAAA

SEQ.ID.NO. 41      HG1014943N1      PLT00014330F01.contig.a  
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SEQ.ID.NO. 42      HG1014944N1      PLT00014330F03.contig.a  
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GAGCTCCAAGAGATTCTCAACTGGCTGAAGGATACACAAGATGTCTTATTTGTGTGTAC  
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SEQ.ID.NO. 43      HG1014945N1      PLT00014330F03.contig.b  
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SEQ.ID.NO. 44      HG1014946N1      PLT00014330F04.contig.a  
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SEQ.ID.NO. 45      HG1014947N1      PLT00014330F04.contig.b  
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 ACCTCTCCTTGTTAG

SEQ.ID.NO. 46      HG1014948N1      PLT00014330F05.contig.a  
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 AAATTAGGCTGGTGTGCTGGCACATGCCTGTAG

SEQ.ID.NO. 47      HG1014949N1      PLT00014330F13.contig.a  
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 CCTCTCCGCCCCAGCTGGAGCCGGGAAGCAGCGGGGCTAA



SEQ.ID.NO. 48      HG1014950N1      PLT00014330G21.contig.a  
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SEQ.ID.NO. 49      HG1014951N1      PLT00014330G21.contig.b  
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SEQ.ID.NO. 50      HG1014952N1      PLT00014330H05.contig.b  
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SEQ.ID.NO. 51      HG1014953N1      PLT00014330H06.contig.a  
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SEQ.ID.NO. 52      HG1014954N1      PLT00014330H12.contig.a  
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 GGGCGCAGAGCCGACCGTGA

SEQ.ID.NO. 53      HG1014955N1      PLT00014330H12.contig.b  
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SEQ.ID.NO. 54      HG1014956N1      PLT00014330H14.contig.a  
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TTCACGATCATGCATGGTAACGTTTAA

SEQ.ID.NO. 55      HG1014957N1      PLT00014330H14.contig.b  
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SEQ.ID.NO. 56      HG1014958N1      PLT00014330H18.contig.a  
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SEQ.ID.NO. 57      HG1014959N1      PLT00014330H18.contig.b  
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SEQ.ID.NO. 58      HG1014960N1      PLT00014330I11.contig.a



CGCGCCCGAGCCGAACGGGTACCGACCGTACCCCGCGATCTTCCTCGAGGGGGGCGCCG  
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SEQ.ID.NO. 62      HG1014964N1      PLT00014330I13.contig.b  
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SEQ.ID.NO. 63      HG1014965N1      PLT00014330J10.contig.a  
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SEQ.ID.NO. 64      HG1014966N1      PLT00014330J10.contig.b  
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SEQ.ID.NO. 65      HG1014967N1      PLT00014330J14.contig.a

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SEQ.ID.NO. 66      HG1014968N1      PLT00014330J14.contig.b

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SEQ.ID.NO. 67      HG1014969N1      PLT00014330J15.contig.a

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SEQ.ID.NO. 68      HG1014970N1      PLT00014330J21.contig.a  
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SEQ.ID.NO. 69      HG1014971N1      PLT00014330J21.contig.b  
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SEQ.ID.NO. 70      HG1014972N1      PLT00014330K01.contig.a  
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SEQ.ID.NO. 71      HG1014973N1      PLT00014330K08.contig.a  
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SEQ.ID.NO. 72      HG1014974N1      PLT00014330K08.contig.b  
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SEQ.ID.NO. 73      HG1014975N1      PLT00014330K09.contig.a  
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SEQ.ID.NO. 74      HG1014976N1      PLT00014330K09.contig.b



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TAG

SEQ.ID.NO. 75      HG1014977N1      PLT00014330K15.contig.a  
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SEQ.ID.NO. 76      HG1014978N1      PLT00014330K15.contig.b  
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SEQ.ID.NO. 77      HG1014979N1      PLT00014330K24.contig.a  
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SEQ.ID.NO. 81      HG1014982N1      PLT00014330M02.contig.b  
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SEQ.ID.NO. 128 HG1015030N1 PLT00014333C21.contig.b



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SEQ.ID.NO. 148	HG1015048N1	PLT00014333G02.contig.b
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 GCAACAACAATCCTTTCCCTCATGCATACITCTCTAATTACAAATGA

SEQ.ID.NO. 186 HG1015084N1 PLT00014333O10.contig.a  
 ATGCTGTGTTTCGTGATAATATATACAGTGGTTGCTGGAGAAAGGAACTTCCCAGAGGG  
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 CTAACGTGTGATTACTGCAGGCTACAAACTTATTTGCCCTCATGGAATAG

SEQ.ID.NO. 187 HG1015085N1 PLT00014333O17.contig.a  
 ATGTTTGACCAGGTGCAGTGGCTCAGCCCTGTAAATCCCAGCACTTTGGGAGGCAGAGGC  
 AGCCACCTCTCAACAACCCATGAAGTGTCTGGAGCTCCACCCTCTGCTCAAGAGGAACGG  
 CACATGACCCAGGCCTATGCCAATCAGCAATTCACATTCCTCATGTTGTTGTTCAAGCTT  
 TGTATCTGGGTACACCTGTTTCCCTCTGAGACATGA

Appendix B

SEQ.ID.NO. 188 HG1014903PIPLT00014330A02.contig.a  
MHVHVHCSTIHNSKIDSTQMPINDALDEENMVYLHHGILCSRKREQDNVLCKDVGGAGS  
HYPQQTNTGTENQTPHVLTYKWEPNNENT

SEQ.ID.NO. 189 HG1014904PIPLT00014330A02.contig.b  
MMHWMKKIWIYITMEYYAAVKGNKIMFFARTWVELEAIIILSKLTQEQTKHHMFSLISGS  
PTMRTHRHREGNNTHLGLSWGWAREGEH

SEQ.ID.NO. 190 HG1014905PIPLT00014330A08.contig.a  
MRFPISLHPHQNLLSVFFILDVLEGVEWYLIIVLICISLRTNAFEHFFMCLLAICISL  
EKCLFKSFVHFLIGLSFYCCMV

SEQ.ID.NO. 191 HG1014906PIPLT00014330A08.contig.b  
MFIQILCPFFNWIVFLLHGISSLYILDTSLLLVIPFANIFSHSVRCLLTFLMCPLKHKS  
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SEQ.ID.NO. 192 HG1014907PIPLT00014330A17.contig.a  
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VNFSKI

SEQ.ID.NO. 193    HG1014908PIPLT00014330A20.contig.a  
 MTYLEIFIQRIYYSIFQCATATTACFWSECSATNIAILLGKRSAGCWIVEVAHI

SEQ.ID.NO. 194    HG1014909PIPLT00014330B02.contig.a  
 MQVHFECNKAHKSPDFVYMTLNLLSDESSVIRFQSSLAQARKWACMTDQAQSGRRPTC  
 CVLSCFPPIAQEDKSSVLGEAKLFS

SEQ.ID.NO. 195    HG1014910PIPLT00014330B02.contig.b  
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 HCVSQDGLNLLAS

SEQ.ID.NO. 196    HG1014911PIPLT00014330B04.contig.a  
 MRIPELGORKQAGRKMGTCGAGGPAGGDLQLGDPQHSTCPALCLVQGCNLPPPASPRHLR  
 APPGEGLVVHTQHLCSLERMGCETPDASQLPSLERLVEWHISLGGSLPRVPSAPAVHAVG  
 PHPSGKRSLLAWVVFPIRNCQVLGLDGLEFPISVGEKGIV

SEQ.ID.NO. 197    HG1014912PIPLT00014330B04.contig.b  
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 PEFPELGWARGPLMRRLPGASQPQSLIAGPDQSRGERPCSLPADRDL

SEQ.ID.NO. 198    HG1014913PIPLT00014330B05.contig.a  
 MRSNLITDILHSNRYITLYSRILRQQPYKVIDAETGPKSKNNLFRVFQPSGEGETWVC

LKPIFTIFFSYDTLPTINE

SEQ.ID.NO. 199    HG1014914PIPLT00014330B11.contig.a  
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NIRLQVHI

SEQ.ID.NO. 200    HG1014915PIPLT00014330B13.contig.a  
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SEQ.ID.NO. 201    HG1014916PIPLT00014330B13.contig.b  
MEFYRSDILTEVYCKIRYSLREKRRQFRGQVERKYTDKVCRSAQGSEAVSWKT

SEQ.ID.NO. 202    HG1014917PIPLT00014330B18.contig.a  
MLFFPSHSILTLSILRSQSCREAAFPWQPIICVIVPCCTIELDTGAMLCLEMYSKASK  
GLPQSPLLKKPQFY

SEQ.ID.NO. 203    HG1014918PIPLT00014330B18.contig.b  
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SEQ.ID.NO. 204    HG1014919PIPLT00014330C06.contig.a  
MFVVCGMFCYCGPSCWTQGHWPAPRHHPSLTLLLSWNTVVPHCPSKATSRHLHLLTPCFFH  
SMVKFFSYQEAGQVQWLTPVPALWEAETGRSFEVRSSRPA

SEQ.ID.NO. 205	HG1014920PIPLT00014330C06.contig.b	MTVSRAPHVLDPRWAEATKGDPARVKGGCRQARAEPCRMRLRARAPPSTGSPSELSIDWT NHPQC
SEQ.ID.NO. 206	HG1014921PIPLT00014330C12.contig.a	MEFRRRAQPWGIYDPWFPSRKLQRKKRAGQPMRERRQRKRKGDGRKGN AFFRTQEKQH QKWAFLPV
SEQ.ID.NO. 207	HG1014922PIPLT00014330C14.contig.a	MQLSNKPSQPGLKVSNSATIKAILCHDQEHRAWSQTDKGLRLMKDCQVTFWSLLHSLEIR TWSFKL
SEQ.ID.NO. 208	HG1014923PIPLT00014330C18.contig.a	MPGQRGPAPASGWKGREQRGGSYSGPPGQSPGVGKQRAKRRPLLSSLTPRPEPKVQVPQ AQSP
SEQ.ID.NO. 209	HG1014924PIPLT00014330C18.contig.b	MSTLSSAGPQLEGAIQSSFPSPAELQRWGGCRAQQCLGRGLCAFCGCGHGHQFRKILNGL SID
SEQ.ID.NO. 210	HG1014925PIPLT00014330D03.contig.a	

MTPLRWYLCTCLFKATFSFLSWTVTDPLYPKNTPLDFRAGCLAYTPGSGLSQGACACH  
CNKEEKTSHGKGLTPSYNQNTQLHREGTPLSHFGSHIHHSVTPTQAGSQVSGVLVLTRAK  
RTKQDPRSPFKH

SEQ.ID.NO. 211      HG1014926P1PLT00014330D03.contig.b

MCISLLRFHMYSFIMRIFFLHPMTIVLIAALKYLTADNNILDILGIAFNAYVLSVCVWITF  
LCFFSCLLNFKIVF

SEQ.ID.NO. 212      HG1014927P1PLT00014330D05.contig.a

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SEQ.ID.NO. 213      HG1014928P1PLT00014330D05.contig.b

MGFPILVSQFRSWYCLSISYPLLGNKLLPNLAASNNNKHVLCHVCFRTLGVA

SEQ.ID.NO. 214      HG1014929P1PLT00014330D07.contig.a

MVCVIPDLFSCIKNIRMNLKVYCHRKSTDQNPQDFKSSGKSGKKLALTSNSTAYKDKGG  
SILSFSIIGPYTLRFCWGVANSCLS

SEQ.ID.NO. 215      HG1014930P1PLT00014330D10.contig.a

MFLVEMGFHHVGQAGLQLLTSSHTLASASLSAGITGTSHRAWPGICIFASNPGHLSAHQS  
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SEQ.ID.NO. 216      HG1014931PIPLT00014330D10.contig.b  
MNSLLTLVLFVCLFSPFLVKCANILKSQENHMHVISHSPLWPASAKMTFCHSYSIHFCHAT  
PDIHTCSFWSIFK

SEQ.ID.NO. 217      HG1014932PIPLT00014330D12.contig.a  
MRKMTFLSADGGGNKNHRDCDFLMVLWVMVEFKLTTKFLVTCFLVFTENILFFETESLTV  
SPRLECSGVMCRLLTATSASWIIQVILMRQPPSSWDYRPPRPANFCIFSIDGVSPC

SEQ.ID.NO. 218      HG1014933PIPLT00014330D12.contig.b  
MEFHVVDQAGLELLASSDPPVSASQAGITGISHHTQPKTFFIMIQVIYQTRNY

SEQ.ID.NO. 219      HG1014934PIPLT00014330D13.contig.a  
MKATMSLLNKSFIKRNIYQGLPCHIDPLKVRSEVEAALMFLNLIIQQKKSKDILTSIDF

SEQ.ID.NO. 220      HG1014935PIPLT00014330D15.contig.a  
MANKHMKKCSFPLVIKECKSKYEIVLYFTRTGMAVIEKTDNDNKYWRGGEEFRNSLVVFQK  
INIESSYNLTIPLDIYPRAMRTYVHTKVCTF

SEQ.ID.NO. 221      HG1014936PIPLT00014330D15.contig.b  
MYDHGLKKLFFFFFFFFFETPCFVAMLGCSGAILAHCNHLHLPGRDSPASASRVAGTTGT

RHHAQLFVFLVEMGFHHVGDGLDLTTS

SEQ.ID.NO. 222    HG1014937PIPLT00014330D17.contig.a  
MGCPSIAEMHQGHISHLLCLGCPICIYQRKPWTPTRGASMRECMPLYKFTPTSEKRPQL  
MLPLPEQCEQLCRFGSTPVTWALJWFGCPTQISS

SEQ.ID.NO. 223    HG1014938PIPLT00014330E04.contig.a  
MLDFTHRSGFRKKQDASAVALYTITLKDILRSCFCFISPYQLPPCCTLQNFGNKT

SEQ.ID.NO. 224    HG1014939PIPLT00014330E14.contig.a  
MMLETHQDCTLEAAVTSEERCPLLWLVKRNQVRLPERFGPLQPATAGLAMPSWNRNRD  
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SEQ.ID.NO. 225    HG1014940PIPLT00014330E14.contig.b  
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SEQ.ID.NO. 226    HG1014941PIPLT00014330E24.contig.a  
MHFGGHIQPISTTIYLGSDFTMENLFSLPQDRQDWKTFPGAAWESPAHQGTGCLSSH  
PTQARELTPESQGWAAQQPPCSPLAPAVSHLTGPRGQGSFHSNYLSYIYTLPK

SEQ.ID.NO. 227    HG1014942PIPLT00014330E24.contig.b



MYTYIYVCISDYKCTYMYIRFHIYCYLFICNEMSQKRNNKKKKRKKKKKKKKRKEKK

RK

SEQ.ID.NO. 228 HG1014943PIPLT00014330F01.contig.a

MESWIAPPFA YEHGYIFSTIFHELKIRLVILHIFFLIYFWIFLLFQGPRVWSDMIELTVG

MNQRQGA YKVSRA TIHC

SEQ.ID.NO. 229 HG1014944PIPLT00014330F03.contig.a

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ELQEILNWLKDTQDVLVCHL FVLLMTPADTTLASSGNKCCCLQHL

SEQ.ID.NO. 230 HG1014945PIPLT00014330F03.contig.b

MEGDSFELCCRSAIAYAVCKVTPSLGASVPFCSPFLDVPCCSLRSFSASSLMWLCSVFC  
LMPQALVPATK

SEQ.ID.NO. 231 HG1014946PIPLT00014330F04.contig.a

MNMLYIALLLPPLTMLEESPT EGCLHRTHTTWSGNSITKTLTYHTYYGCMGNRLGTCTY  
SQTTYVCDPGNNQLYVCYDPKFPSPGEWFEIRAVKRRSPLKPNQGPSLLLRDYFSVF

SEQ.ID.NO. 232 HG1014947PIPLT00014330F04.contig.b

MRNAIQNRLALDYLLALEGVVCGKFNL TNCCLEIDDNGKA MEITARMRKLAHVPVQTW  
KGWSPDSLFGGLVFIFLRVQDFNRSGSGHIRKLPNTPLSLTSPC

SEQ.ID.NO. 233 HG1014948PIPLT00014330F05.contig.a  
MSVIQTLWEADAGRSLEVRSSRPARTWQNPVSTKNMKKKLGCAGTCL

SEQ.ID.NO. 234 HG1014949PIPLT00014330F13.contig.a  
MGRSRCPSPRRLLVSLARAVSPVAGKTGRPASSVPTTQPLRPQLEPGSRRG

SEQ.ID.NO. 235 HG1014950PIPLT00014330G21.contig.a  
MVPHHLSSDRLLFLTSVMFPHTDSLFSMDTLSTTALEKLGPLLGSHPKPHTNF  
LRSSPSAPFSANTPTSILSEHPVLVSTVLLSPFQAVPKVIIQGYVSFHLKCIIPPLLGSQ  
ISFPGPHHLINFLNIRIINNDTSVF

SEQ.ID.NO. 236 HG1014951PIPLT00014330G21.contig.b  
MYICILCIYNDEKTDIFTLTYTVVCVYLWTKPIFSMNCLFLLISYRICMLHNI

SEQ.ID.NO. 237 HG1014952PIPLT00014330H05.contig.b  
MNSRFESVTKCLPKEKSPRLGFYADFYPIYKEKQTPILLKLPKIEEGILPNSLYKASI  
TLISNQDKDTTKGENYRPFLMNTDGKILSIILPSQI

SEQ.ID.NO. 238 HG1014953PIPLT00014330H06.contig.a  
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SEQ.ID.NO. 239    HG1014954PIPLT00014330H12.contig.a  
MGLLPQAGLAGLGGWGAARPSLKAPRGAVGRAQGRSVVLLVPSCGAGSRAVHGRGRDPA  
PRGRSPAPARPWPRPRPGGGAQSRP

SEQ.ID.NO. 240    HG1014955PIPLT00014330H12.contig.b  
MRRSRTRSLADKPVESAILERVPEAPGFQFRLLTRQPCDLGQLNVRKLGSVFSIGLMSV  
RGSRKGGWPAGNLRSM

SEQ.ID.NO. 241    HG1014956PIPLT00014330H14.contig.a  
MLVIIRLSCKTALYGLRDYCLSYPLLGTIRLSVSPEVVDQINVPVRHKPREGKVCLNS  
FTIMHGNV

SEQ.ID.NO. 242    HG1014957PIPLT00014330H14.contig.b  
MSMCVCYNFSFKKSTPGSLSTFLNFSFAHNFRFTEELQNSSYMPFTQIPLVLFYMTMVY  
LSKLRK

SEQ.ID.NO. 243    HG1014958PIPLT00014330H18.contig.a  
MALLCHIFLFTVLLPPFTLTSLPPCCCTTSSSSYQEVLWRMWLPRNIDVPSYRGFSKGGP  
TFTTHNHIPLHFRPYISIPVSLTSLSMSLPESKL

SEQ.ID.NO. 244    HG1014959PIPLT00014330H18.contig.b  
MRIKRNHRKRPDTGNNIFDKYEFLFFPLFYFTATPTSYSLSFATKQTSGFVCLFHQGEV

LNVSFNPQSIHSLRG

SEQ.ID.NO. 245 HG1014960PIPLT00014330I11.contig.a

MLSKNIRTCETLFVYTQTHLHIYLYLSIEIYCVVCVCMYLCVCVSLSVLVHLSYNT

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SEQ.ID.NO. 246 HG1014961PIPLT00014330I12.contig.a

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SEQ.ID.NO. 247 HG1014962PIPLT00014330I12.contig.b

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VVAFLF

SEQ.ID.NO. 248 HG1014963PIPLT00014330I13.contig.a

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LYLAYKGGLFRETRMEPFRDMSTL

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FWQGKKVSALWMKLSVRPPLLLTPSTAGAGRFQATGLHRPSMLTCTPAHSLTNWSLPPHLL  
ELTRASKADG

SEQ.ID.NO. 251    HG1014966P|PLT00014330J10.contig.b  
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RCYHPARKDFEKSSVLWAVGSHLHSPRPQRKAVSLCRAEV

SEQ.ID.NO. 252    HG1014967P|PLT00014330J14.contig.a  
MLFSIVGEPFYPPQVHHFILHSGCTILVPANSAQWLQYLLLLTNTCYLLFLDNGHLSRC  
EAYLTPYTKNSKWIGVA

SEQ.ID.NO. 253    HG1014968P|PLT00014330J14.contig.b  
MFATWHSIANYIHGAVQHASRALPSHVAETLHVPPPTPGNHYSLLCVYKFDCFRDLI

SEQ.ID.NO. 254    HG1014969P|PLT00014330J15.contig.a  
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QSVKNIQK

SEQ.ID.NO. 255    HG1014970P|PLT00014330J21.contig.a  
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HSYA VECYAAGRKKGQIDMY

SEQ.ID.NO. 256    HG1014971P1PLT00014330J21.contig.b  
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GGCGTRIT

SEQ.ID.NO. 257    HG1014972P1PLT00014330K01.contig.a  
MALAFFHMSPTNFTELTPTPHTPPPGMEARRDSSIVQKKSGENKKGEGDIAAVWCFIL  
CVCFLPRKKNRL

SEQ.ID.NO. 258    HG1014973P1PLT00014330K08.contig.a  
MCHHVLLVFKFFCRNRISLHCPGWSPSLEQSSCLSLPEWWDYRCEPHTLAFFCLPFNSVC  
CFLTYRGFKFCCSWITLPFVTFEIASIKLNLFPFQIQK

SEQ.ID.NO. 259    HG1014974P1PLT00014330K08.contig.b  
MKSSIFFHINWPFIFLCELSVITFYLLHSNTIQNIILLQLFYFLLTFLFI

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MAKRGQGTVLAIASEGASSKPWQLPHGVGVSVMQKTRTEVQETLAGCMKMPGCDRSLQ  
GWSPCGRTSARAEQKGNVGLKSPHRVPTVALTSGAKKRGL

SEQ.ID.NO. 261    HG1014976P1PLT00014330K09.contig.b

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SEQ.ID.NO. 262      HG1014977PIPLT00014330K15.contig.a

MMPGETHSAAPGTAADLSRCQGCASLQQNLNEYVEALITLKQKIINTDNLLTEYQKKCDD

ILLAFSVEFWN

SEQ.ID.NO. 263      HG1014978PIPLT00014330K15.contig.b

MAGRPQETYNHGRRGSKHILHMVAGERSAERSGEKPLIKPSDLVGTHSLSGEQHWGTAPV

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SEQ.ID.NO. 264      HG1014979PIPLT00014330K24.contig.a

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SEQ.ID.NO. 265      HG1014980PIPLT00014330L01.contig.a

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GISTKTTKKLAGCGGGLLWSQLPWRLREENGVNPGGACSELRSRHCTPTWA

SEQ.ID.NO. 266      HG1015004PIPLT00014330L24.contig.a

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SEQ.ID.NO. 267      HG1014981PIPLT00014330M02.contig.a

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TQTSKMKRKATFRKPSSIQCFFVSSSSPLKGLRMMSSKFDHLGF

SEQ.ID.NO. 268    HG1014982PIPLT00014330M02.contig.b  
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EQIRKQDLSGAMTSSTVDGKEMRGAATC

SEQ.ID.NO. 269    HG1014983PIPLT00014330M08.contig.a  
MYPCVLIQHPLIGENMQCLVFCVSLLRIMASSSIHVPAKDMISFLFVATQYSMVVYMYHV  
FFIQFVIDGHLG

SEQ.ID.NO. 270    HG1014984PIPLT00014330M08.contig.b  
MSSTMVELIYSTNGVKVLLFLHSLASIFCFLTFYFIYLFYLFCTPAWATQ

SEQ.ID.NO. 271    HG1014985PIPLT00014330M15.contig.a  
MGENKGERSETAACARPPLAHSRPAAPRAPPSPSLPRLLLTTPSERPGRESGG

SEQ.ID.NO. 272    HG1014986PIPLT00014330M17.contig.a  
MAAAA VRPVTWTTGLPTTPPRLEDAQRRLGLRGVSQREPRSPGRRRLGAGSLAGRP  
RLPPESAGAPRRPCPVVHRPDPRVPRLGLLRLLTTLNFLSRVTHSSSLGPF

SEQ.ID.NO. 273    HG1014987PIPLT00014330M17.contig.b



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VAGTTGARHHAQMRKLRHREVK

SEQ.ID.NO. 274    HG1014988PIPLT00014330N10.contig.a  
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LSGMSYCQGEGLPRC

SEQ.ID.NO. 275    HG1014989PIPLT00014330N10.contig.b  
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SSRPAAWAT

SEQ.ID.NO. 276    HG1014990PIPLT00014330N12.contig.a  
MSQQSNWEPNSDQQGGIVWLGSDRNLGRKQPHTKDNKKVTWALKDSVKKPETPK

SEQ.ID.NO. 277    HG1014991PIPLT00014330N12.contig.b  
MIKKGKCPLAASTVRLELTEKELLYYLLHSLQRKLEKWVDVIERTCVCGARQTVV

SEQ.ID.NO. 278    HG1014992PIPLT00014330N13.contig.a  
MMTMLPVFVLTLTYCLLFRVYCSYLSKCLTAKQLQAGPSGDVPEEGIAIDDDSSMHVIA  
PEELSAVQDVEVEDSDIDDPDLV

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SEQ.ID.NO. 280      HG1014994PIPLT00014330N22.contig.a

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SEQ.ID.NO. 282      HG1014996PIPLT00014330O03.contig.a

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SEQ.ID.NO. 283      HG1014997PIPLT00014330O07.contig.a

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SEQ.ID.NO. 284      HG1014998PIPLT00014330O07.contig.b

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SEQ.ID.NO. 285      HG1015005PIPLT00014330O18.contig.a

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SEQ.ID.NO. 286    HG1015006PIPLT00014330O18.contig.b  
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SEQ.ID.NO. 287    HG1014999PIPLT00014330P07.contig.a  
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LSNSEGPFRLDRMSKGQSIAPNTLL

SEQ.ID.NO. 288    HG1015000PIPLT00014330P07.contig.b  
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SEQ.ID.NO. 289    HG1015001PIPLT00014330P09.contig.a  
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SSQNAPHLLSGLLTGIGGLAAQHGPPPWETPSLLS

SEQ.ID.NO. 291     HG1015003PIPLT00014330P15.contig.a  
MRLWGECKNLKDKVAQSNQMSIQGRFLSHPLQKSPSRARVNQWKFLALTAHYSYSFINV  
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SEQ.ID.NO. 292     HG1015007PIPLT00014333A03.contig.a  
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SEQ.ID.NO. 293     HG1015008PIPLT00014333A03.contig.b  
MVEAKQKSDLMGTA PGFVCPLESSFLQGHNVKQRVLLFRKLTYSFRPVLLGGKKEGST  
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SEQ.ID.NO. 294     HG1015009PIPLT00014333A06.contig.a  
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PGFIW SCLKVKSLEFLMIPFLYGLQFDRWEFSTLKKTKLLSGNPCPPLTSTQNCFPHSLT  
ARVVKNWDVLLRWA VECHYPQVTTDVLTPSMFR

SEQ.ID.NO. 295     HG1015010PIPLT00014333A06.contig.b  
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SEQ.ID.NO. 296    HG1015011PIPLT00014333A08.contig.a  
MLQFSCTGDVVASQTFTVTAGFKKQMRPFQPKAIWLVFEPQVQFFIDFYHLIVRSAISGL  
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SEQ.ID.NO. 297    HG1015012PIPLT00014333A15.contig.a  
MGASAAACSCPGHGSTKLISVTTSLTVGLDLNMHTGSGTKGADFLQLSQAGVPAQQMVP  
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QSSRQAVPTCHVGWEM

SEQ.ID.NO. 298    HG1015013PIPLT00014333A15.contig.b  
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SEQ.ID.NO. 299    HG1015014PIPLT00014333A16.contig.a  
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SEQ.ID.NO. 300    HG1015015PIPLT00014333A16.contig.b  
MKHFFHSEKFPSICLQSILPPALSIHELLICFLSLQGSSHFPEFYINRIV

SEQ.ID.NO. 301    HG1015016PIPLT00014333B03.contig.a  
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SEQ.ID.NO. 302    HG1015017P|PLT00014333B03.contig.b  
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SEQ.ID.NO. 304    HG1015019P|PLT00014333B05.contig.b  
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SEQ.ID.NO. 306    HG1015021P|PLT00014333B17.contig.a  
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GETHFIPDTSLAERHR

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SEQ.ID.NO. 308    HG1015023P|PLT00014333C02.contig.a

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 EGVLGPLAVPAHRRCARFLAGP

SEQ.ID.NO. 315    HG1015030P1PLT00014333C21.contig.b  
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SEQ.ID.NO. 316      HG1015031P1PLT00014333C24.contig.a  
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MYIYVRMHVHMFEVCIHTKNPYIHCKMKRPMDS

SEQ.ID.NO. 317      HG1015032P1PLT00014333C24.contig.b  
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VHICTMYVVCMIYICLCVYTKHTTYIOK

SEQ.ID.NO. 318	HG1015033P1PLT00014333D07.contig.a
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SEQ.ID.NO. 320      HG1015035P1PLT00014333D15.contig.a

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LTGD

SEQ.ID.NO. 321      HG1015036P1PLT00014333D15.contig.b

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SEQ.ID.NO. 322      HG1015037P1PLT00014333E01.contig.a

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SEQ.ID.NO. 328    HG1015043PIPLT00014333E14.contig.b  
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SEQ.ID.NO. 329    HG1015086PIPLT00014333E15.contig.a  
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PGSRHSPASASRVAGTTGTRHHARLTLFLYFK

SEQ.ID.NO. 330    HG1015087PIPLT00014333E15.contig.b  
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SEQ.ID.NO. 331    HG1015044PIPLT00014333E24.contig.b  
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VEKCLGKLHQRVLLYHDNAPAHSSHQTRGNL

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SEQ.ID.NO. 336    HG1015088PIPLT00014333G09.contig.a  
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SEQ.ID.NO. 342 HG1015053PIPLT00014333I18.contig.b  
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NHF AKMKGWG

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SEQ.ID.NO. 347 HG1015058PIPLT00014333J13.contig.a

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SEQ.ID.NO. 351    HG1015062P1PLT00014333J17.contig.a  
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NVKELELSFIAGMNNENWHNCLGKQFSSS

SEQ.ID.NO. 352    HG1015063P1PLT00014333J23.contig.a  
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SEQ.ID.NO. 356    HG1015067PIPLT00014333K08.contig.a  
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SEQ.ID.NO. 358    HG1015069PIPLT00014333L13.contig.b  
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SEQ.ID.NO. 359    HG1015070PIPLT00014333M01.contig.a  
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SEQ.ID.NO. 363    HG1015074PIPLT00014333M07.contig.a  
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SEQ.ID.NO. 365    HG1015076PIPLT00014333M15.contig.a  
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SEQ.ID.NO. 366    HG1015077PIPLT00014333M15.contig.b  
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# Appendix C

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ATTTCTGTGAATGGCAGATTATGGTT

SEQ.ID.NO. 376 HG1014905N0 PLT00014330A08.contig  
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